SOME CHEMISTRY OF THE NITRO GROUP IN THE CONTEXT OF A POSSIBLE SYNTHESIS OF VITAMIN B_{12}

by

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DOCTOR OF PHILOSOPHY

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To the Memory of my Mother, Iza

PREFACE

The work described in this thesis is a record of research carried out during the period October 1971 to September 1974 in the Department of Molecular Sciences of the University of Warwick, under the direction of Professor J.W. Cornforth, F.R.S., and Dr B.T. Golding. It is believed wholly original except where due acknowledgment is made and has been presented for no other degree.

ACKNOWLEDGMENTS

I thank Professor V. M. Clark for making available the facilities of the Department of Molecular Sciences, enabling the presentation of the research described herein.

The inspiration behind the synthetic approaches towards Vitamin B_{12} is due to Professor J. W. Cornforth, F. R. S., whom I wish to thank for providing stimulating ideas whenever the group met for discussions concerning the project. Professor Cornforth is also thanked for supervising an enjoyable period spent at the Milstead Laboratory of Chemical Enzymology during 1973.

I am grateful to Professors A. J. Birch, V. M. Clark and J. W. Cornforth, who generously made funds available for an enjoyable stay as a Visiting Research Scholar in the Research School of Chemistry, the Australian National University, Canberra from January - June, 1973.

The receipt of a Science Research Council Studentship in conjunction with Shell Research Limited is gratefully acknowledged.

Dr Bernard Golding, who has directed the majority of this work, acting both as mentor and friend, deserves my lasting and sincerest thanks. His stimulating supervision and enthusiasm have helped to make the last three years particularly satisfying, and it is hoped that some of the qualities he instils are reflected in this thesis.

Finally, my thanks must also go to Mrs J. McConnell for typing the majority of this work, and also to Mrs C. A. M. Billing and Mrs J. M. Cole who kindly assisted in the completion of the typing.

ABSTRACT

This thesis describes new nitro-chemistry in studies related to a possible synthesis of Vitamin B_{12} as proposed by J. W. Cornforth. Some of the Vitamin B_{12} intermediates possess special nuclear magnetic resonance properties when examined as their nitronate anions. Some synthetic studies of fluorinated compounds as potential substrates for glyceroldehydrase, an enzyme requiring 5'-deoxyadenosyl cobalamin (Coenzyme B_{12}), are also described.

The Introduction discusses the chemical synthesis of corrins, together with some of the prominences of the Eschenmoser-Woodward synthesis of Vitamin B_{12}. A brief account of the possible synthesis of Vitamin B_{12} proposed by Cornforth is also given. The chemistry of the related B_{12} coenzymes is summarised in a section on the enzymatic reactions requiring a 5'-deoxyadenosyl cobalamin as a cofactor. The current proposals for the mechanism of action of glyceroldehydrase and dioldehydrase are also compared.

The Cornforth approach to Vitamin B_{12} was to utilise the reaction of an anion with a geminal bromonitro or dinitro compound in order to join precursors of ring A and ring D. Chapters I and II describe the synthesis of suitable precursors and models for these radical-anion coupling reactions, and attempts to realise an intermolecular coupling of ring A and ring D intermediates. The synthesis of 4-carboxamidodiethylmalonyl-3-methyl-3-(2', 2'-bromonitroethyl)cyclohexanone ethylene ketal and 4-(2', 2'-dinitro)n-butoxycarbonyl-3-(2'-nitroethyl)-3-methyl cyclohexanone are described together with attempts to use these intermediates for intramolecular coupling purposes. In chapter I, the attempted coupling of hindered geminal halogenonitroso compounds with nitronate anions is also described.

Attempts to incorporate an intramolecular Michael addition into the synthesis, for the linking of ring A and ring D precursors are the subject of chapter III. The synthesis of amide derivatives of Hagemann's ester (4-ethoxycarbonyl-3-methyl cyclohex-2-enone) containing a nitro-enamide residue as the 4-substituent, to examine this scheme was unsuccessful. The corresponding hydrazide analogues, for example, 3-methyl cyclohex-3-ene-1-one ethylene ketal 4-carboxylic acid 3'-nitrobutylidene hydrazide, are more readily accessible and some reactions are described.

In chapter IV, interesting long range deshielding effects are noted in the ^{1}H n.m.r. spectra of the nitronate anions derived from 4-alkoxycarbonyl-3-methyl-3-(2'-nitroethyl) cyclohexanones, ring A precursors for coupling studies. The deshielding effect associated with the nitronate anion is a useful probe in conformational analysis, and shows the axial conformer of 2-substituted cyclohexane nitronate anions to be preferred. Also the ^{13}C n.m.r. spectra of several nitronate anions show large solvent dependent chemical shift difference which correlate with differing electronic distibution in the anions in solvents of different protonating ability.

Chapter V is an account of the synthesis of potential substrates and inhibitors for the enzyme glyceroldehydrase. 3, 3, 3-Trifluoro-1, 2-propanediol is a substrate for the enzyme and is converted to 3, 3, 3-trifluoropropionaldehyde isolable as its 2, 4-dinitrophenyl hydrazone. Attempts to synthesise authentic 3, 3, 3-trifluoropropionaldehyde and the effects of the CF_3 group are also discussed. The reaction between 3, 3, 3-trifluoro-1, 2-propanediol and glyceroldehydrase is readily monitored by ^{19}F n.m.r. allowing an examination of reaction intermediates. The synthesis of several bromopropanols and some preliminary investigations as to their reactions with glyceroldehydrase are also noted.

CONTENTS

General Arrangement of the Text and Experimental Methods

The chapters in this thesis represent either differing synthetic or practical approaches, in an area of study related to vitamin B_{12}. For ease of reference a separate experimental section is appended to each chapter. Diagrams, schemes, figures and tables are numbered separately for the chapter in which they appear, although the references run consecutively throughout the work.

The following details describe the experimental methods used.

Melting points (m.p.): Melting points were measured on a Reichert heated microscope stage and are uncorrected.

Infrared spectra (i.r.): Infrared spectra were recorded using a Perkin-Elmer 257 grating spectrophotometer calibrated against a polystyrene film. Solids were recorded as 1% solutions in the solvent indicated, in 0.2 mm path-length cells with sodium chloride windows, or as nujol mulls. Liquids were measured as thin films between sodium chloride plates. The absorption maxima are given in wavenumbers (ν cm^{-1}) followed by the intensity designated as: w (weak), mw (medium weak), m (medium), ms (medium strong), s (strong), vs (very strong). The additional qualifications br (broad) or sh (shoulder) may be added.

For most compounds only absorptions at wavenumbers higher than 1500 cm^{-1} are given.

Nuclear magnetic resonance spectra (n.m.r.): ^1H n.m.r. spectra at 60 MHz were recorded using a Perkin-Elmer R-12 spectrometer, for dilute solutions in the solvent indicated, with tetramethylsilane (TMS) as internal reference. For aqueous solutions, sodium 3-(trimethylsilyl)-propanesulphonate was used as internal reference. The chemical shifts of resonances are given downfield relative to the internal reference (δ 0.00). Resonances are followed by their multiplicity: S (singlet), d (doublet), t (triplet), q (quartet), sx (sextuplet), m (multiplet), dd (double doublet) and where appropriate the preceding qualification br (broad) is used; the spin-coupling constant (J) in Hz; and the integration (nH).

The suffix (100 MHz) indicates that spectra were recorded on either a Varian HA-100 or a JEOL MH-100 spectrometer. Spectra at 90 MHz were recorded on a Bruker WH 90 using the pulse Fourier transform method:

^{13}C n.m.r. spectra were recorded at 22.63 Mz on a Bruker WH 90 spectrometer as described in the appropriate experimental section (IV).

Ultra-violet spectra (u.v.): Ultra-violet spectra were recorded on a Pye-Unicam SP 800 spectrophotometer in methanolic solution using quartz cells. Absorption maxima are given in nanometers (nm) followed by the molar extinction coefficient (ϵ). The qualifications br (broad) or sh (shoulder) may be added. (MeOH/HCl) indicates that 2 drops of aqueous hydrochloric acid was added to the methanolic solution and likewise (MeOH/NaOH) indicates 2 drops of aqueous sodium hydroxide.

Mass spectra (m.s.): Mass spectra were measured on an AEI MS 902 spectrometer by the University of Hull Mass Spectrometry Service, or on an AEI MS 9 by the Physico Chemical Measurements Unit, Harwell. Where a full fragmentation pattern is given the m/e value is followed in bracketsby the intensity as a percentage of the base peak peak (B). The molecular ion is signified by M^+ and for precise mass measurements the molecular formula having the best correlation is given.

Elemental Analyses: Combustion analyses were performed by Dr. F. B. Strauss (Oxford) or Alfred Bernhardt (W. Germany).

Refractive Index (n_D^t): Refractive indexes were measured on a Hilger-Watts refractometer at t^oC using a sodium lamp as the light source.

Chromatography: Gas-liquid chromatography (g.l.c.) was performed on a Honeywell F and M instrument using the column and temperature indicated. The injection port was at ca. 260^oC and the detector at ca. 280^o.

Thin layer chromatography (t.l.c.) was performed on plates prepared from Machery, Nagel and Co. Silica gel G/u.v. 254 eluting with the solvents indicated. An adsorbent thickness of 0.25 mm was used for 5 x 20 cm plates, and for microscope slides (prepared by spreading) a 0.15 mm thickness. Plates were developed by examination under ultra-violet light (λ 254 nm) and/or by exposure to iodine vapour.

Preparative layer chromatography (p.l.c.) was performed on 100 x 20 cm plates using 0.5 mm or 0.75 mm thicknesses of Merck silica gel PF, 254, the mixture being applied with a Burkhard SA 100 applicator. When only small quantities of mixtures were available, they were applied manually to 20 x 20 cm plates.

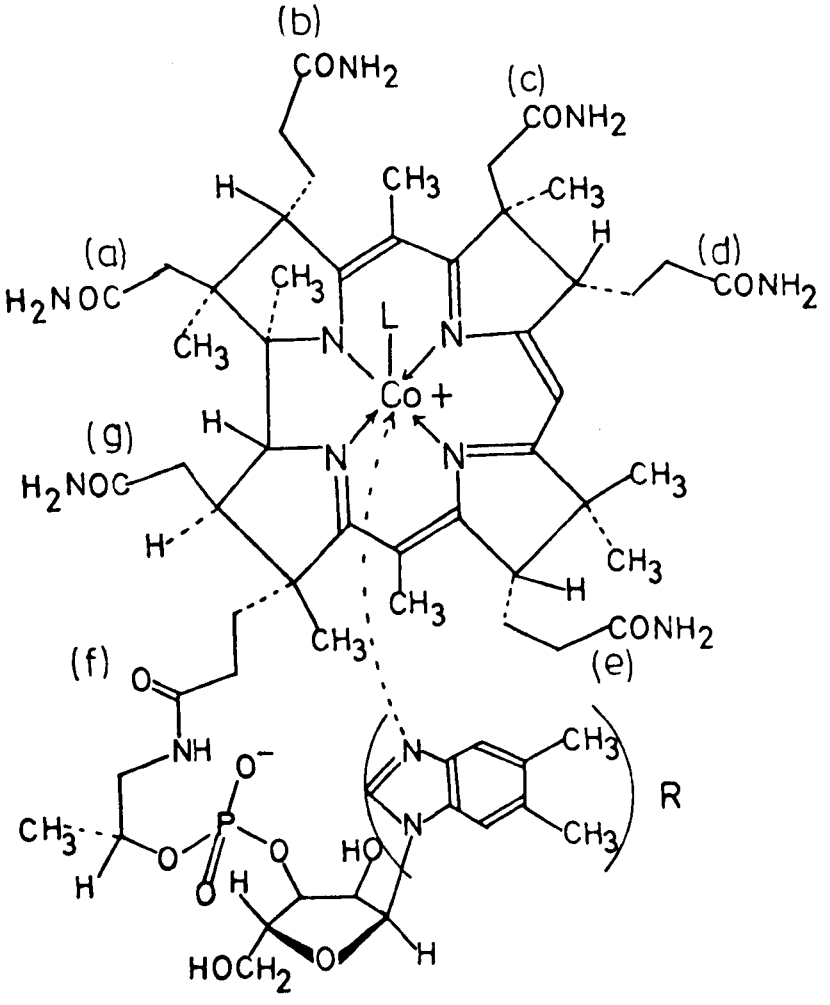
Column chromatography used Fisons or B.D.H. (100-200 mesh) silica gel in a ratio of ca. 50 : 1 w/w to the mixture. For small scale separations, and purifications of mixtures containing components of widely differing Rf values, the 'silica gel-filtration' technique was used - Machery, Nagel and Co. silica gel N in a glass sinter funnel was subjected to moderate suction after applying the mixture, while eluting with the solvent indicated.

Solvents and Reagents : Solvents were either of analytical reagent quality or purified to a similar standard according to methods described[145]. Spectroscopic grade solvents were used where appropriate, and if necessary purified further. Reagents were generally of the best grade commercially available, and where necessary were purified sufficiently for the intended purpose.

General Procedures : Routine drying of solutions was carried out with anhydrous magnesium sulphate (prepared by heating dried magnesium sulphate at 150°C for 24 hrs) unless specified otherwise. Routine evaporation of solvents *in vacuo* was carried out using a Büchi rotary evaporator at water pump pressure. A Virtis freeze-drier was used for the removal of traces of volatile materials from products.

Abbreviations : Abbreviations used are explained above or in the text where appropriate. In the experimental sections certain common reagents are referred to by their chemical formulae. mM is used for millimoles.

INTRODUCTION



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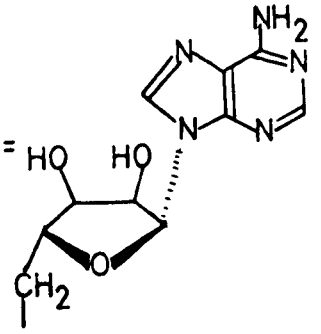
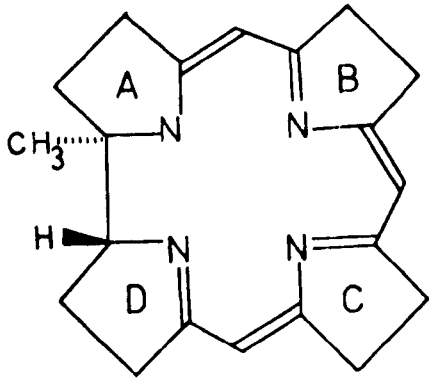
(2a) L =  = L'



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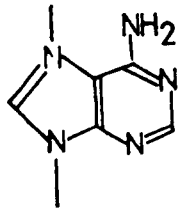
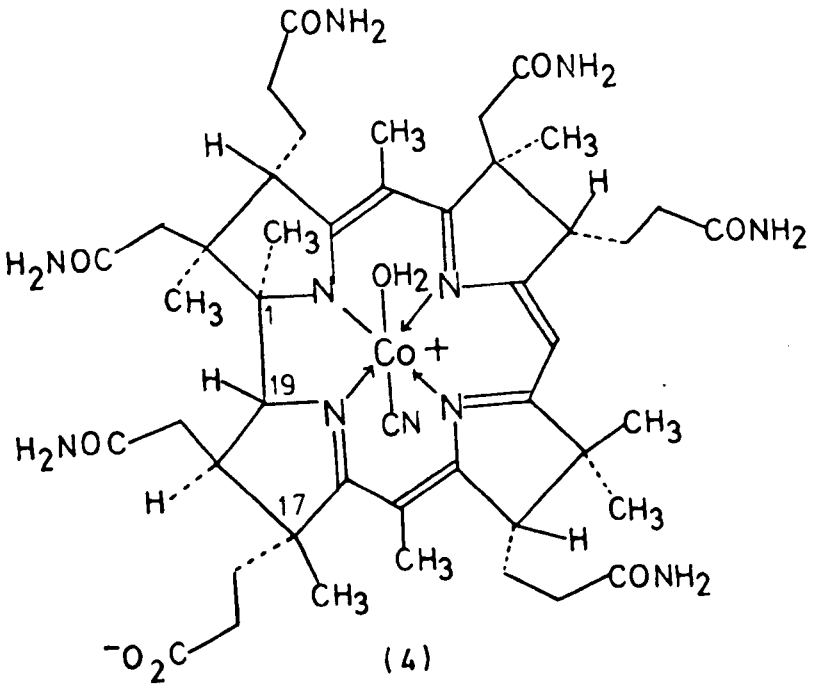
(2b) L = L' R = 

FIGURE 1.

Vitamin B_{12} was first isolated from natural sources in 1948,[1] and since then research in this area has been in a continuous state of development. The first goal achieved was the establishment of the structure of vitamin B_{12} as α-(5,6-dimethylbenzimadazoyl)cobamide cyanide (cyanocobalamin) (1). A considerable effort was necessary, using a combination of degradative chemistry, and X-ray crystallography, before the structure was finally revealed.[2] It is now suggested that cyanocobalamin does not normally occur in nature, being instead an artefact, the cyanide ligand arising from the isolation procedure. Later in 1958[3] a coenzyme form of vitamin B_{12} was isolated from the anaerobic bacterium Clostridium tetanomorphum, and its participation in the isomerisation of glutamate to 3-methyl aspartate catalysed by an enzyme from the same bacterium was demonstrated. X-ray crystallography[4] again resolved the structure of the coenzyme and the first naturally occurring substance containing a carbon-cobalt bond was discovered.

The structures of the principal cobalamins are shown in Figure 1. In vitamin B_{12} the cobalt atom is bound by coordinate linkages to the four nitrogen atoms of a partially hydrogenated tetrapyrrole macrocycle, called a corrin ligand. The cobalt and the nitrogen atoms of the corrin nucleus are approximately coplanar. Complexes which contain the corrin ligand (all related to (3)) are known as corrinoids. The partially reduced macrocyclic tetrapyrrole is also related to uroporphyrin III, but the substituent methyl groups control the level of reduction. An important difference between the corrin and porphyrin structures is that the former incorporate a direct link between the A and D rings whereas porphyrins have methylene bridges linking all the pyrrole rings. The other noteworthy features are the ligands axial to the cobalt. In vitamin B_{12} one axial ligand is cyanide and the other is α-5,6-dimethylbenzimidazole bound to cobalt at N-3. The latter molecule is also bound at N-1 via a chain containing 3'-ribosephosphate linked (via O) to isopropanolamine which is joined (via N) to a propionate side chain at C-17 of the corrin nucleus.

There are two important coenzyme forms of vitamin B_{12}. Benzimidazole-B_{12} coenzyme is identical in structure to vitamin B_{12}, except the cyanide ligand is replaced by 5'-deoxyadenosine attached at C-5' to the cobalt atom. Adeninyl cobamide coenzyme was the first of the coenzymes isolated and also has 5'-deoxy-



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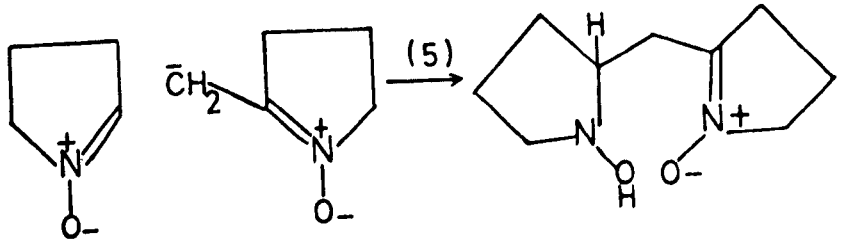
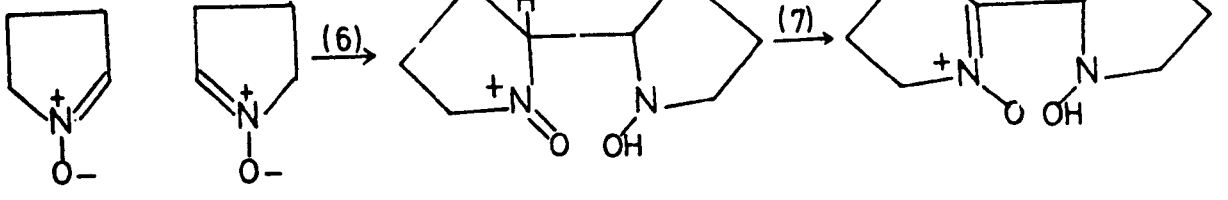
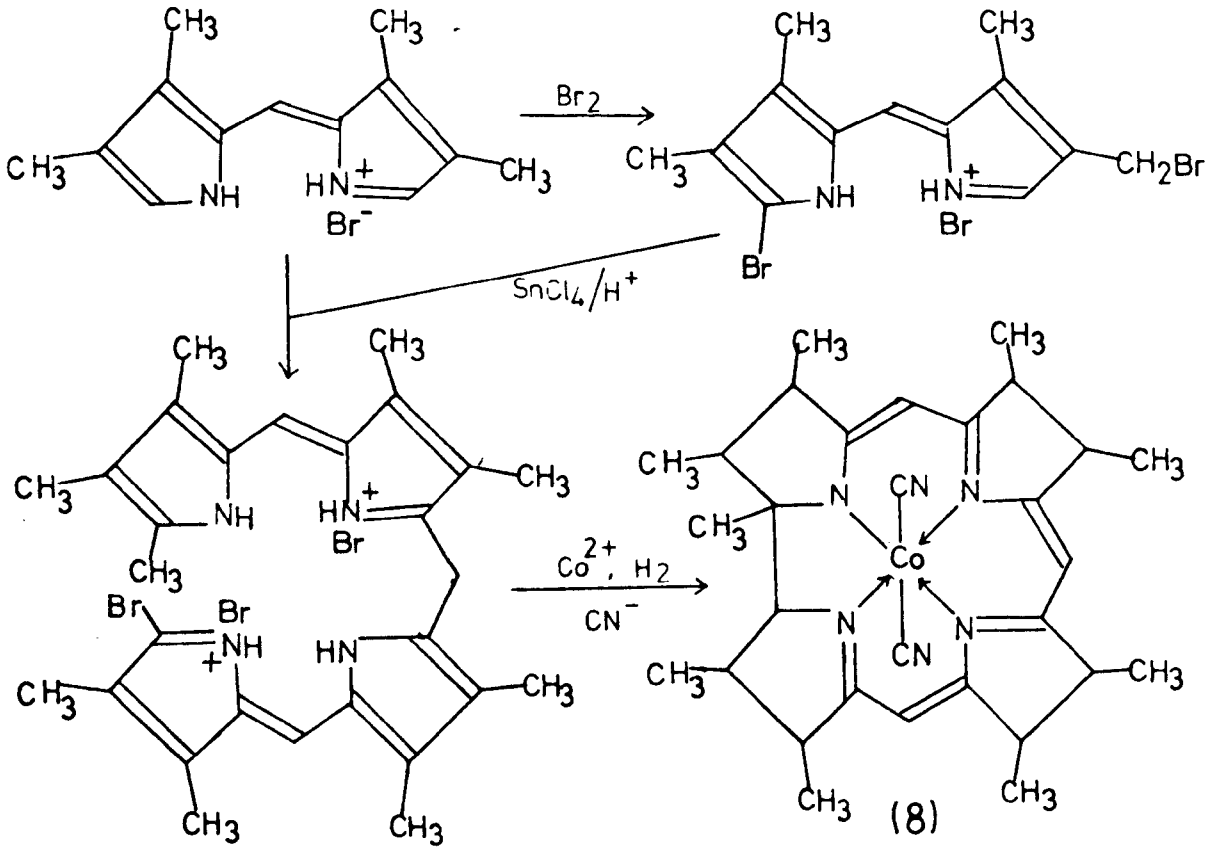
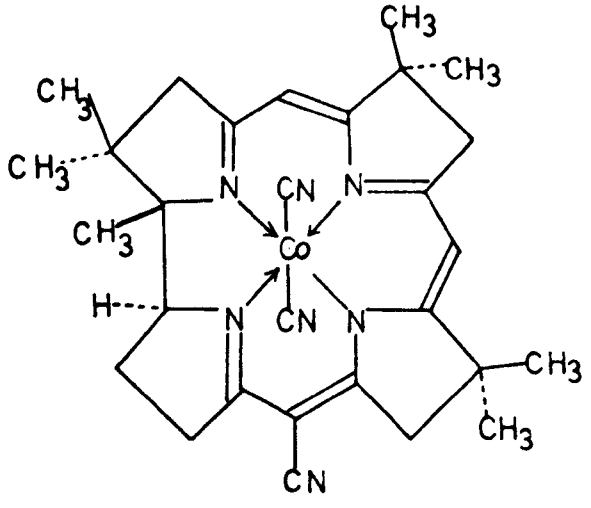






SCHEME I



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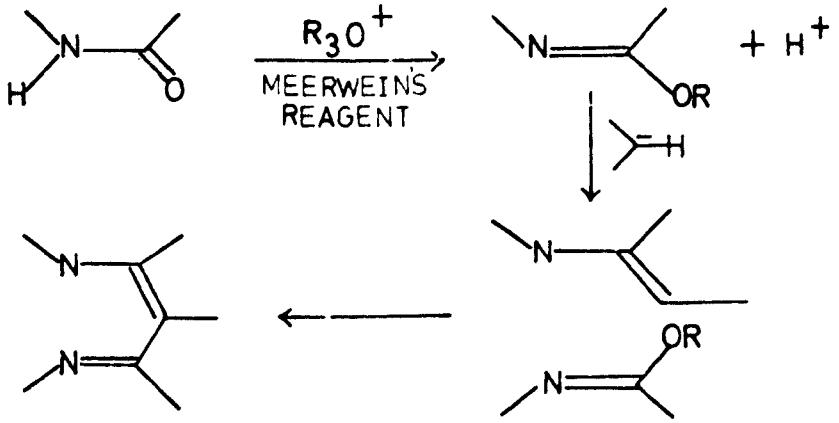

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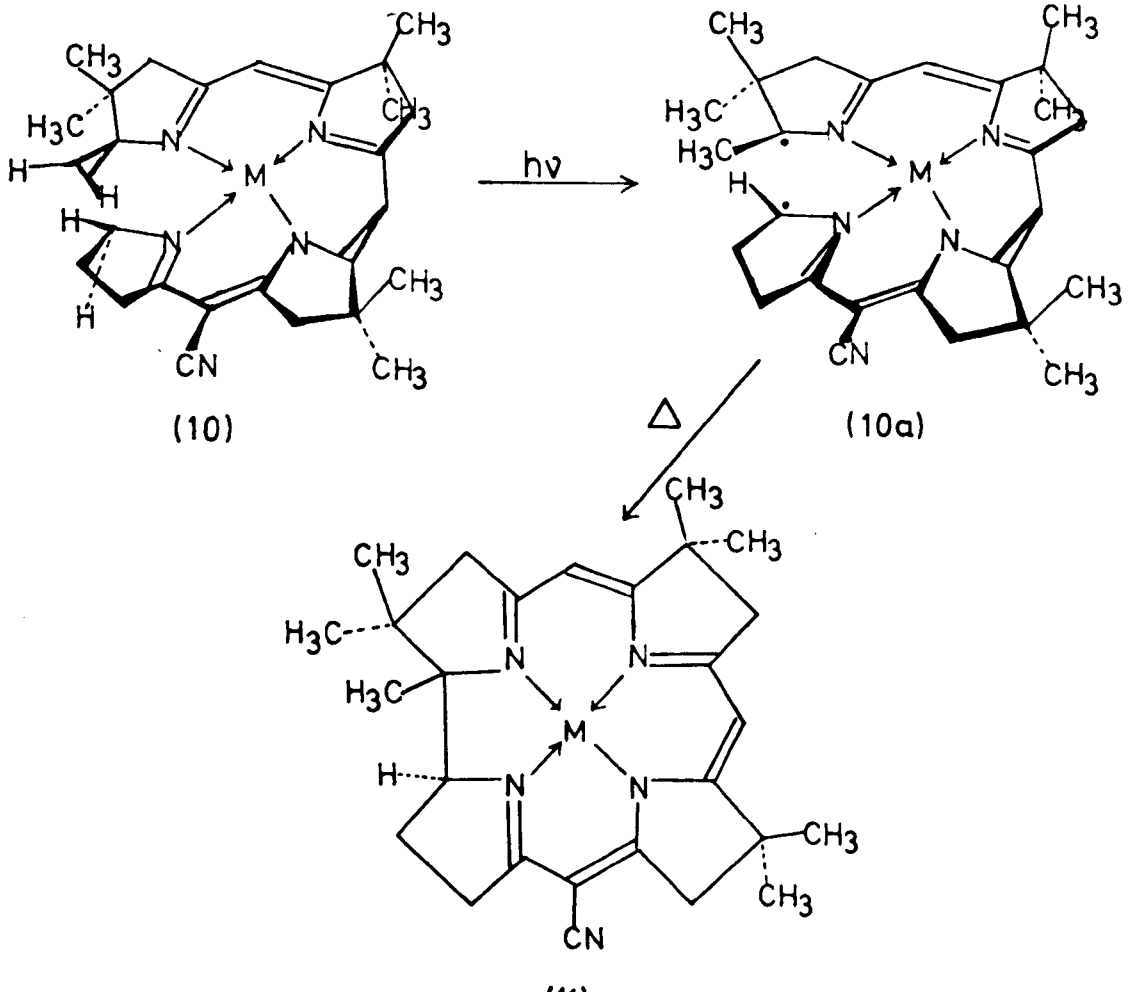
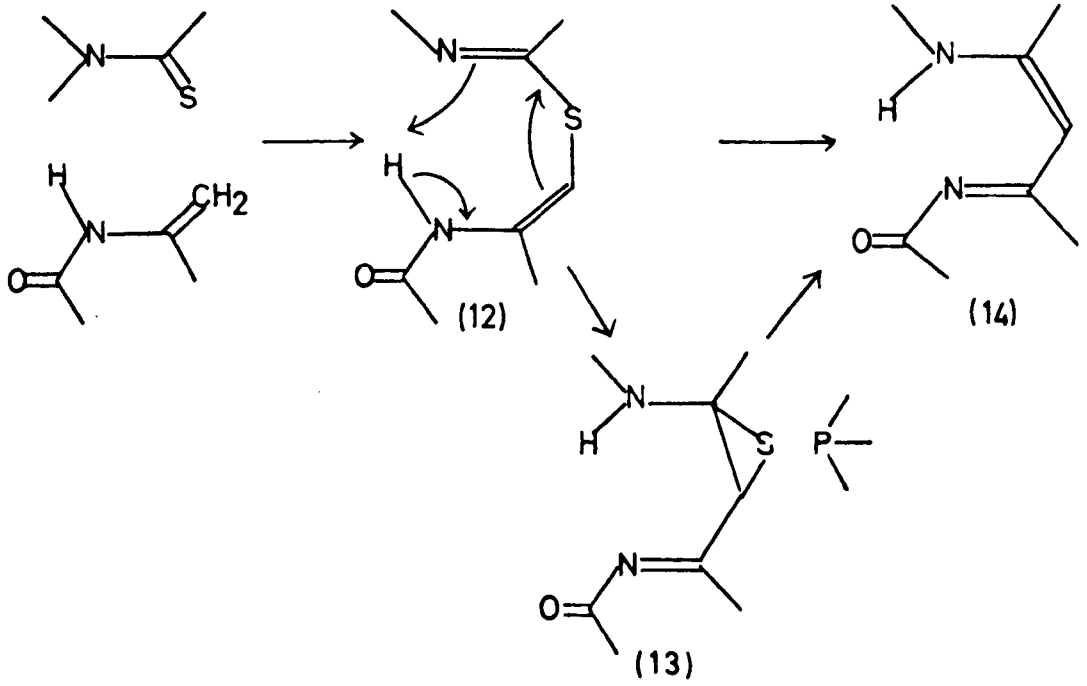




SCHEME III

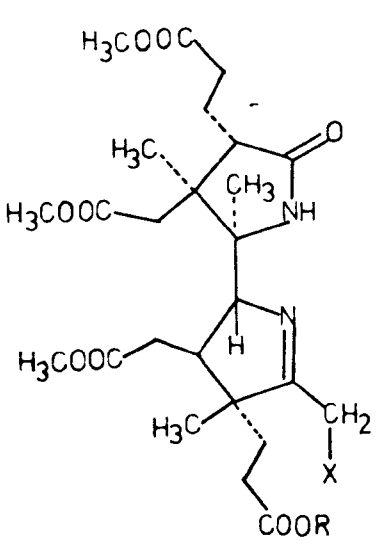
adenosine as an axial ligand. The other axial ligand, however, contains an adeninyl moiety in place of the 5,6-dimethylbenzimidazoyl grouping. In cobyric acid (4), the simplest naturally occurring cobalamin, there is no nucleotide present, and a bare propionate side chain is the substituent at C-17. Water and cyanide are the two axial ligands. Cobyric acid contains all the essential features of the vitamin's corrinoid nucleus and was used as a precursor for the partial synthesis of vitamin B_{12}.[5] Consequently cobyric represented the immediate goal of the total chemical synthesis of vitamin B_{12}, and the B_{12} coenzymes since the latter are available from the vitamin.

Chemical Synthesis of Corrins

The intriguing structure of vitamin B_{12} induced several groups to embark on chemical syntheses of corrinoids. The foundations of chemical corrin synthesis began with the efforts of Todd[6] and his collaborators. 1-Pyrroline N-oxides were used such that either a new methylene bridge could be constructed as in transformation (5), or the two pyrrolines could be directly linked, as in (6) to give (7). This approach has the advantage of using reduced pyrroles but was not developed for further coupling.

Corrins and porphyrins have been prepared from the cyclisation of linear tetrapyrroles by Johnson[7] and coworkers (Scheme I). Although this approach bears some resemblance to the biogenetic pathway (the porphyrin and corrin pathways are closely related), the corrin (8) produced requires tetramethylation before this approach can be incorporated into a synthesis of cobyric acid. There is likely to be little stereochemical control during these latter steps, such that after the introduction of each methyl group producing a new asymmetric centre, then the correct diastereoisomer must be resolved.

Eschenmoser's efforts[8,9] resulted in the synthesis of the cobalt corrin (9) which was formed by an A/B cyclisation. The concept behind this approach depended on the reaction of imino esters with a carbanion or an enamine to construct the vinylogous amidine system, which is the characteristic structural element of the corrin chromophore (Scheme II). Another novel approach[10] to corrins was pioneered by Eschenmoser's group. Instead of an A/D to B/C type



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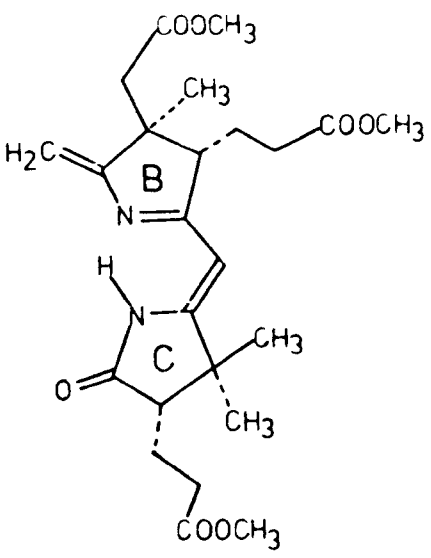

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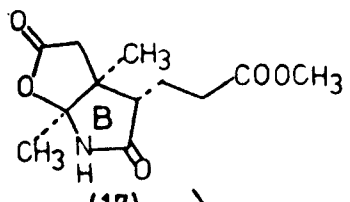

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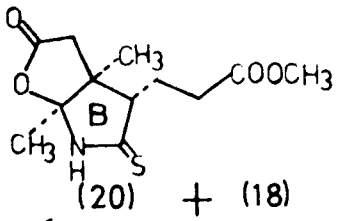

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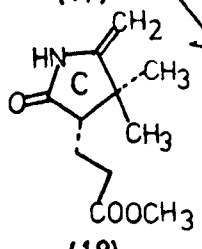

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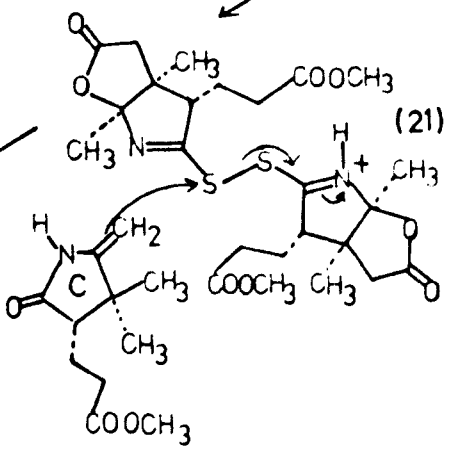

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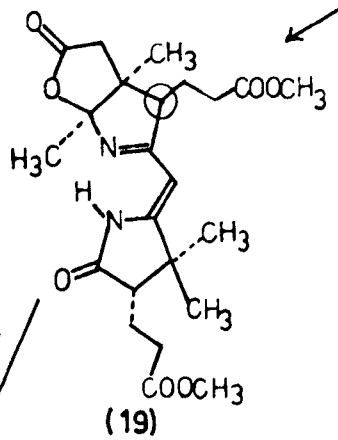

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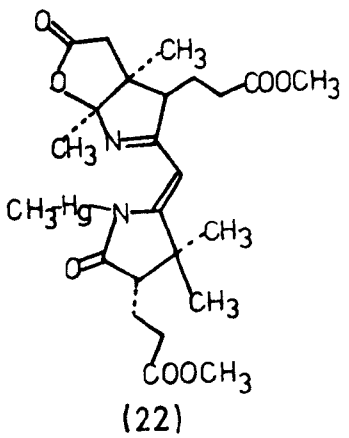
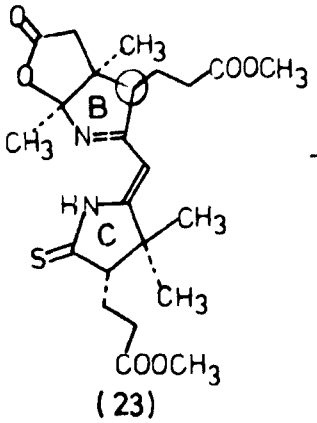


(22)

$\xrightarrow[2.\ H_2S]{1.\ (CH_3)_3O^+}$



(23) + EPIMER

cyclisation, a photochemical A/D cyclisation was used, and although this made it similar in strategy to Johnson's cyclisation of linear tetrapyrroles, mechanistically it is very different. From a study of models of complexes such as (10), one of the ring D methylene hydrogens at position 19 lies directly below the exocyclic methylene group of ring A. This geometric configuration is satisfactory for a light induced sigmatropic 1,16-hydrogen transfer from C-19 in ring D to the exocyclic methylene carbon at ring A. The primary product (10a) has a 15-centre, 16 electron π system and proceeds to a trans-corrin complex(11)via an exothermic antarafacial electrocyclic 1,15 (π - σ)-isomerisation. The Woodward-Hoffmann rules[11] predict that the hydrogen transfer would have to be induced photochemically; the experimental results are in accord with this.

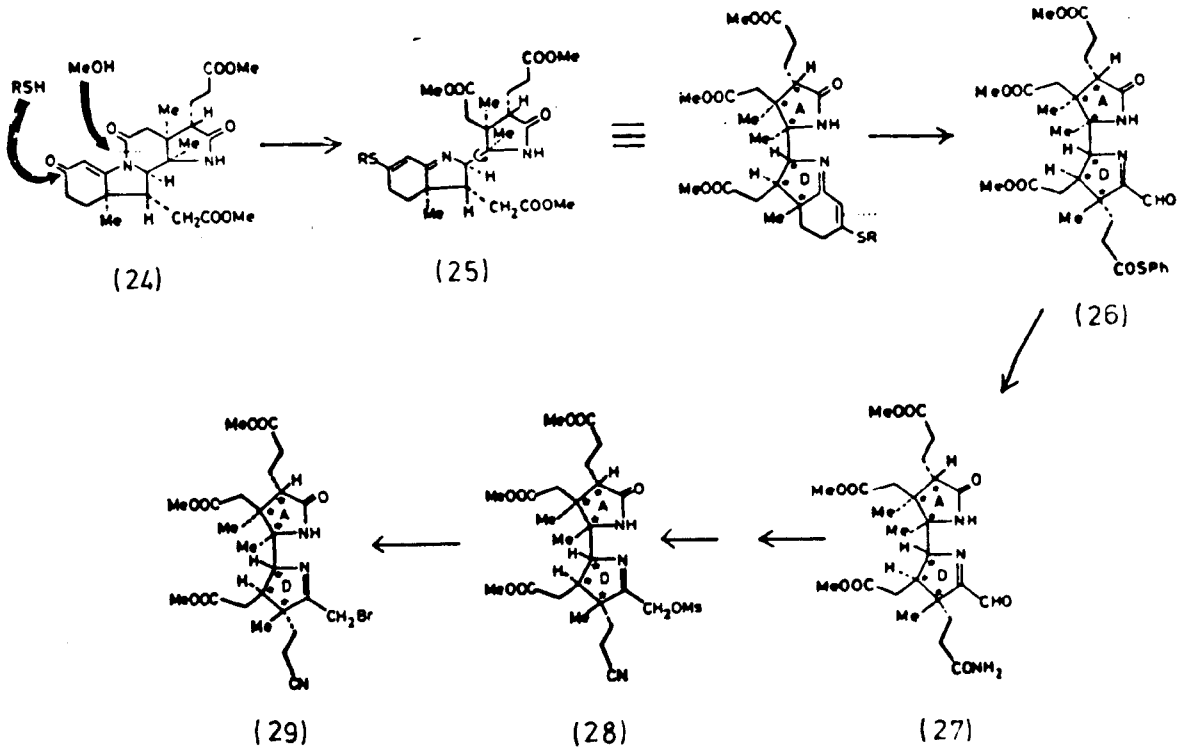
Another new development in corrin synthesis resulted directly from studies involving precursors for the B/C component for vitamin B_{12}. Formally the method is an intramolecular version of the iminoester-enamine condensation (Scheme III). A thiolactam is oxidatively coupled with an enamide using benzoyl peroxide to give the sulphur bridged intermediate (12). In the presence of a phosphorus thiophile, sulphur extrusion occurs, possibly via the episulphide (13), resulting in the vinylogous amidine system (14). The method was successfully employed in the synthesis of seco-corrinoid complexes (10) starting from simple monocyclic precursors.

The Eschenmoser-Woodward Synthesis of Vitamin B_{12}

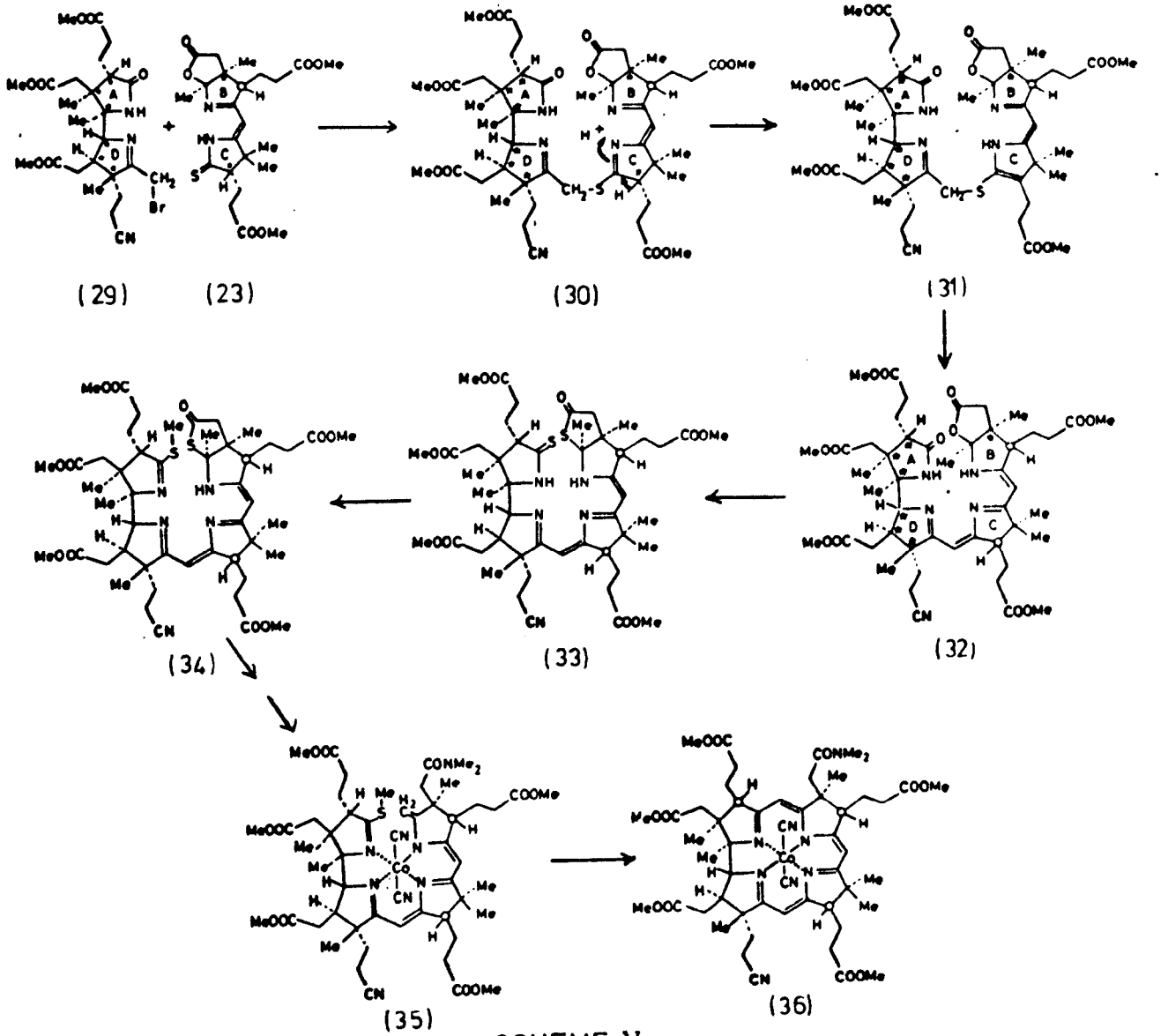
The magnitude of work carried out during the various studies in this area prevents a detailed discussion. Some of the highlights and difficulties in the synthesis are briefly discussed here, as much of the completed synthesis has now been well reviewed.[12]

The collaborative approach towards cobyric acid was for the A/D portion (15) to be synthesised in the Harvard laboratories and the B/C component (16) at the E.T.H., Zürich. The failure to synthesise the B/C precursor (19) by an iminoester-enamine condensation procedure (Scheme II) using (17) and (18) even after a variety of structural modifications and reaction conditions, led to the application of the sulphur-contraction method outlined earlier (Scheme III).

Oxidation of the thiolactam (20)with one equivalent of benzoyl peroxide in the presence of the enamide (18), followed by sulphur extrusion by triethylphosphite



SCHEME IV

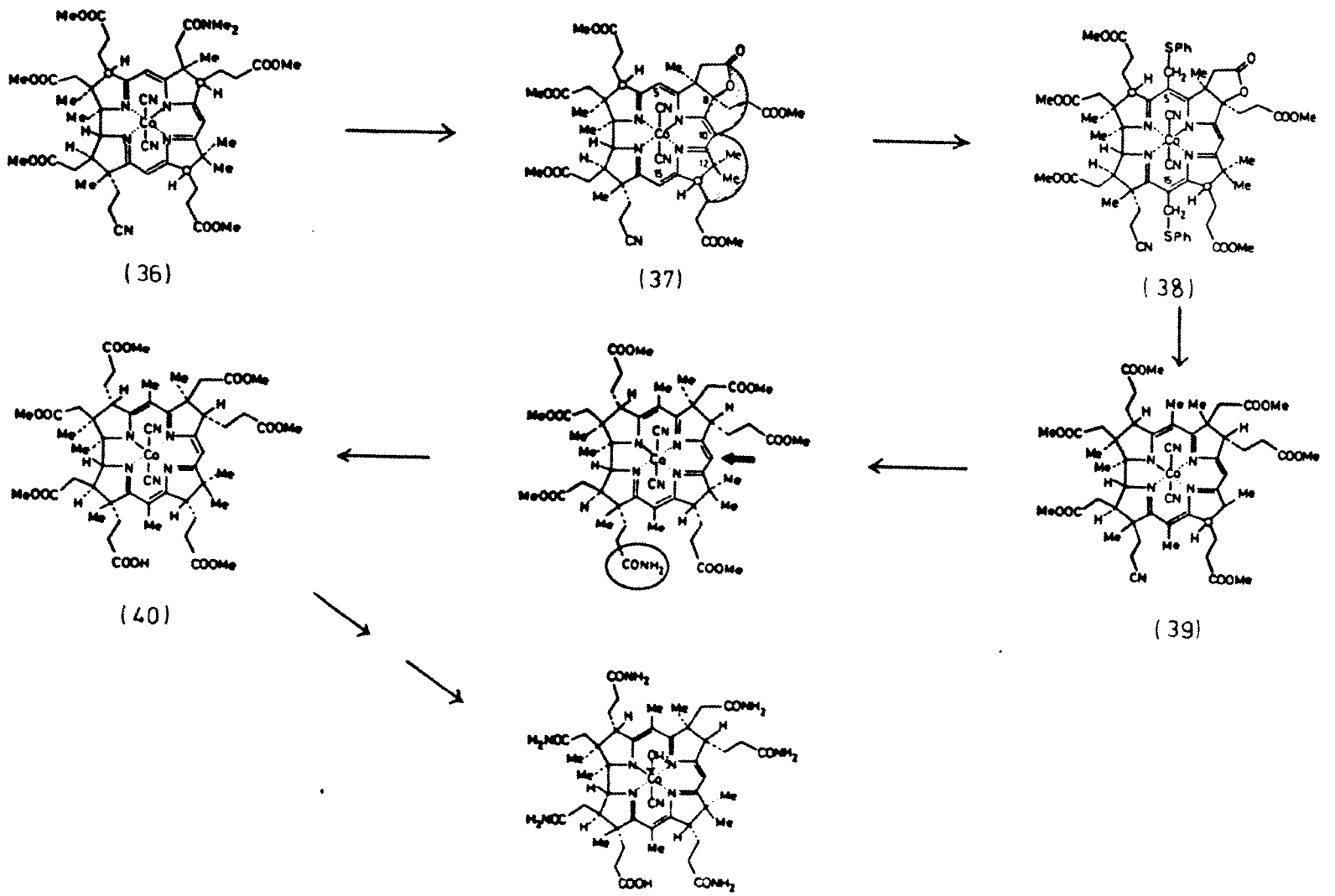


SCHEME V

gave the B/C precursor (19). The bis-imidoyldisulphide (21) was identified as the intermediate, a trace of acid catalysing the electrophilic substitution at the methylene carbon; the equivalent of thiolactam liberated was then oxidised and recycled. Studies using models indicated that an iminoester-enamine condensation would not be suitable for the D/C coupling of the A/D and B/C components. Consequently the thiodextrolin (23) was synthesised so that a sulphide contraction could be incorporated into the D/C coupling. The synthesis of (23) resulted in a mixture of epimers differing in configuration only at the ring B asymmetric centre. The isomers could be separated but equilibration occurred very readily in subsequent steps, so the epimeric mixture was used.

The key intermediate used for the preparation of the A/D component has been termed β-corrnorsterone (24). Its synthesis has been outlined by Woodward.[12a] The steps from β-corrnorsterone to the final A/D component are shown in Scheme IV. This approach cleverly solved the problem of the differentiation of the side chain at ring D from the other six ester side chains. Cleavage of (24) by methanol alone gave a product with all methyl propionate side chains. However, by using a mercaptan (R = Et, Me, Bu^t, Ph) containing a small proportion of methanol, the methanol cleaved the lactam, whereas the mercaptan was incorporated in the unsaturated system of (25). Ozonolysis of (25) (R = Ph) followed by ammonolysis of the thioester (26) in liquid ammonia gave the aldehyde-amide (27), with a propionamide side chain at C-17, and methyl propionate side chains in the other positions. The cyanobromide (29) was the final A/D component with the stereochemistry defined at each of the six asymmetric centres. The transformations from (27) are also shown in Scheme IV.

Several methods have been discovered for the linking of the A/D and B/C components and also for cyclisation to the macrocycle. One of the more direct routes is shown in Scheme V. A diastereoisomeric mixture of thiodextrolin (23) was coupled with the cyanobromide (29), giving the Type I thioether (30). However, (30) readily equilibrated to the Type II isomer (31) and this was thought initially to be a dead end. Treatment of (31) with tris-β-cyanoethyl phosphine in sulpholane overcame this difficulty giving the cyanocorrigenolide (32). A key transformation was the conversion of this lactam lactone (32) to the dithiocyanocorrigenolide (33). Methylation of (33) using trimethyloxonium fluoroborate gave the S-methyl derivative


(36)
(37)
(38)
(39)
(40)
COBYRIC ACID

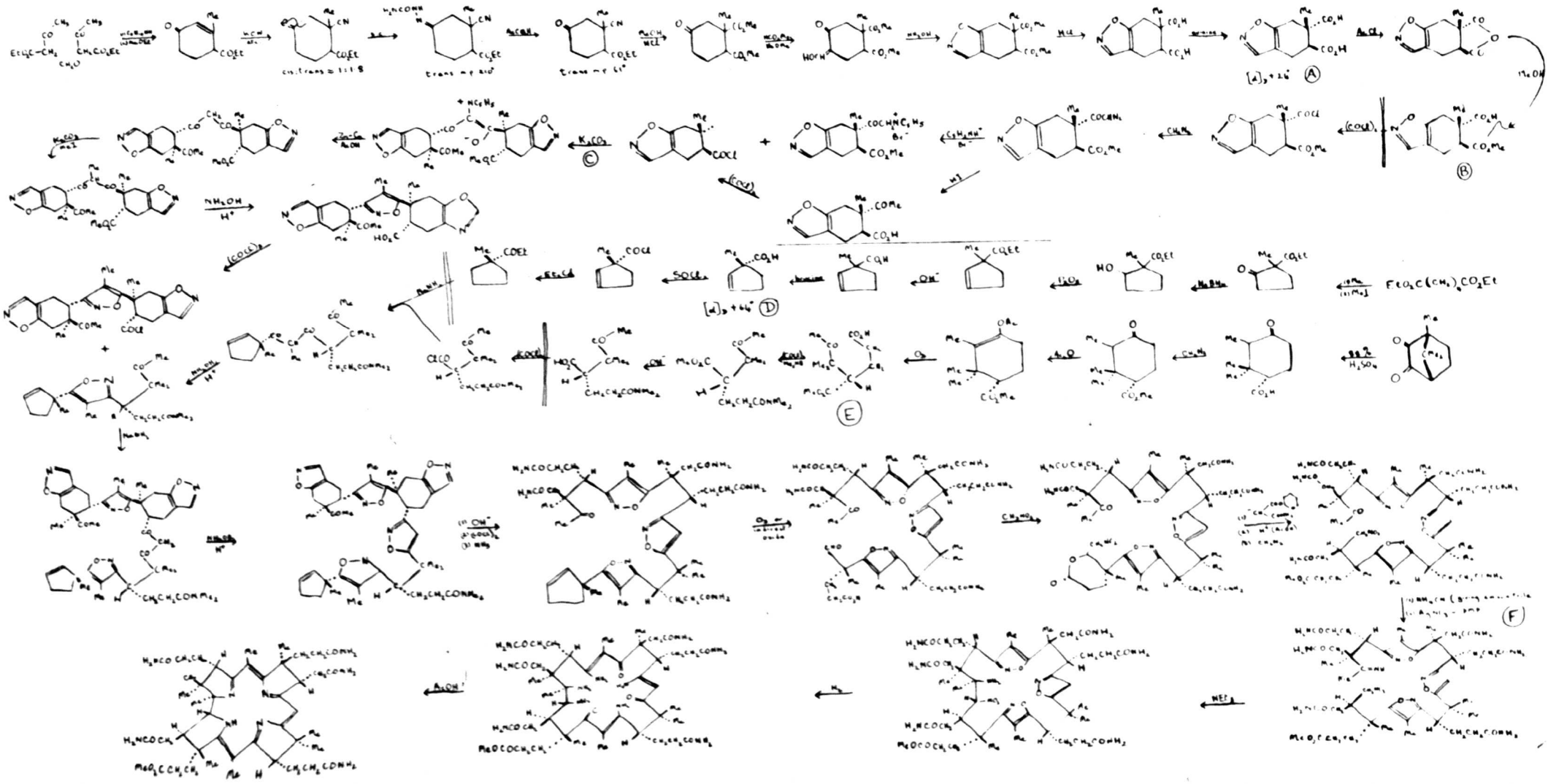
(34), the thiolactone grouping of which was cleaved by dimethylamine in methanol giving a dimethylamide group and the important exocyclic methylene. (34) was then directly reacted with a cobalt halide in tetrahydrofuran resulting in (35) incorporating the cobalt atom. The cobalt atom holds the nucleus together and facilitated cyclisation under various basic conditions yielding (36) : bis-norcobyrinic acid abdeg pentamethyl ester c dimethylamide f nitrile. Due to the ready equilibration at three asymmetric centres, the corrinoid produced was a mixture of isomers epimeric at C-3, C-8 and C-13. Other methods of closing the macrocycle have been developed, including the use of zinc as a metal template, prior to its replacement by cobalt. This and other alternatives are discussed by Woodward in his final account of the total synthesis.[12c]

The seemingly simple stages left in the synthesis, i.e. the introduction of methyl groups at C-5 and C-15, without methylating C-10, and the differentiation of the ester side chains, were, in fact, tedious steps. The dimethylamide function introduced into (36) allowed direct cyclisation to the lactone (37) - a reaction which did not occur with the corresponding ester. Preparation of the lactone created full substitution at C-8 and, together with the two methyl groups at C-12, helped to render C-10 inaccessible to substitution. Subsequently thiophenyl methyl groups were introduced at C-5 and C-15 only. Desulphurisation using Raney nickel gave the required methyl groups, and simultaneously the carbon-oxygen bond of the lactone was reduced to give a free carboxyl group. This was esterified with diazomethane yielding an ester side chain in (39) which was a mixture of isomers separable by high pressure liquid chromatography. Treatment of the nitriles (39) with concentrated sulphuric acid gave a propionamide side chain at C-17 which allowed the differentiation from other side chains. Woodward found that nitrogen tetroxide in carbon tetrachloride at 0° C in the presence of sodium acetate was effective for the deamination of the C-17 propionamide residue to the f-acid (40). Attempts at deamination using nitrous acid and nitrous esters readily nitrosated the corrin nucleus at C-10. The final step to totally synthetic cobyric acid involved ammonolysis of the six carbomethoxy groups using liquid ammonia. Again, high pressure liquid chromatography was necessary for the purification, but a product so obtained had properties identical to cobyric acid from natural sources.

Of course, during a project of this magnitude, several avenues were explored

SCHEME VI

B12 PROJECT July '62



simultaneously. The photochemical cyclisation that had earlier been employed in corrin syntheses[10] was also successful when applied to the problem of cobyric acid synthesis. In the approaches to bis-norcobyrinic acid abdeg pentamethyl ester c dimethylamide f nitrile (S) (36), when using a zinc derivative, the formation of the A/D bond was non-stereospecific and led to a mixture of compounds. However, using a cadmium derivative in the photoreaction, stereospecific cyclisation occurred almost exclusively to give materials of the natural series. An important technical development during this work was that of high pressure liquid chromatography; in many cases where compounds had identical spectroscopic properties, they could only be resolved by liquid chromatography.

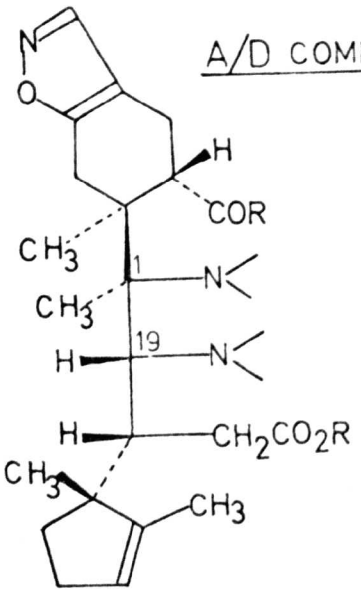
The Cornforth Approach to Cobyric Acid

In 1962, Cornforth proposed[13] a synthesis of vitamin B_{12}, essentially different in strategy from the approach by Eschenmoser and Woodward. The original concept drawn by Professor Cornforth is reproduced in Scheme VI. An important feature of the synthesis is the use of isoxazoles as corrin precursors. Stevens[14] has since used isoxazoles as intermediates in the synthesis of semi-corrins. A drawback to this approach, however, was the cyclisation step forming the 1,19 bond to give the metal free corrin. The formation of this highly hindered bond is left to a very late stage in the synthesis, and also by a reaction which is reversible. Together with the steric factor, there is a high entropy factor against bond formation.

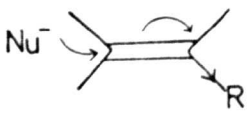
After some work synthesising and resolving precursors, the strategy was changed to the synthesis of an A/D component, and then subsequent coupling with a B/C component, as in the Eschenmoser-Woodward scheme. The proposed A/D component (41) was designed so that it could be incorporated into the earlier scheme using the same ring B and ring C precursors. The objective was to stereospecifically join the carbon atoms which would ultimately become C-1 and C-19 in cobyric acid, and to have each bearing a nitrogen substituent.

Initial studies[15] investigated the use of the attack of a nitronate anion on an electron deficient double bond (the Michael reaction (42) or the Meyer reaction (43)). Ring D precursors which could be synthesised were (47) and (48) and were

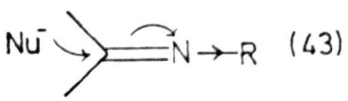
A/D COMPONENT



(41)

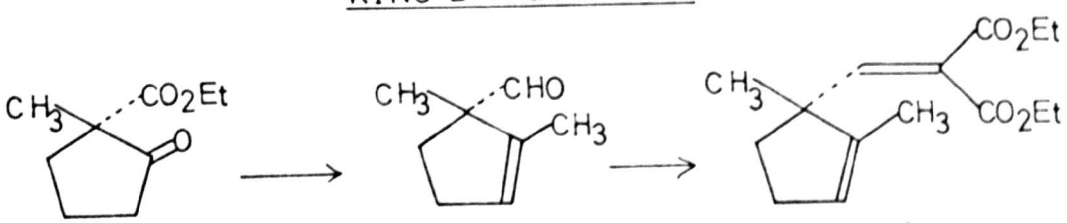


(42)

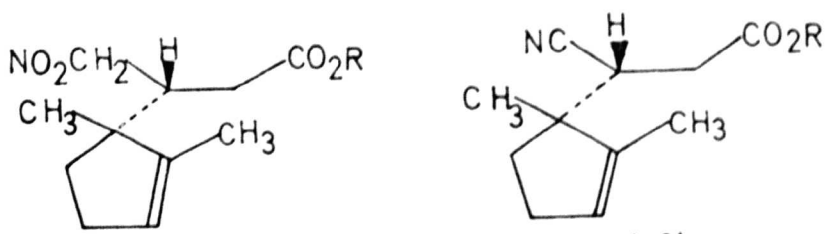


(43)

RING D PRECURSORS

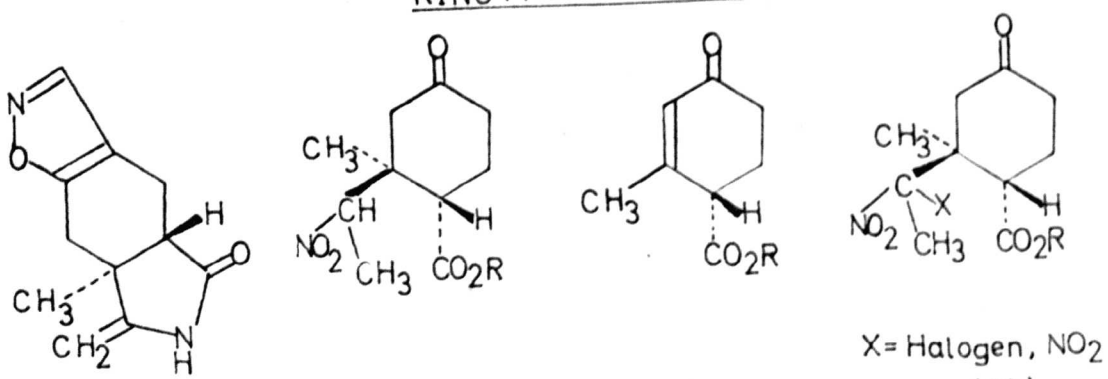


(44) (45) (46)



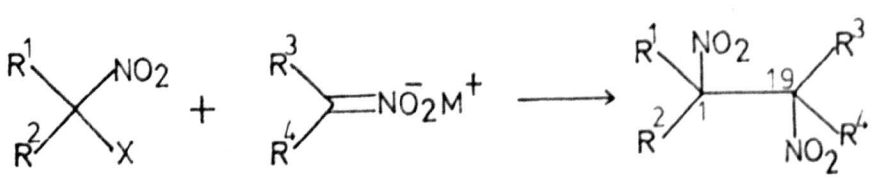
(47) (48)

RING A PRECURSORS



(50) (49) (51) (52)

X= Halogen, NO_2



SCHEME VII

derived from (46). Ring A precursors obtainable were (49) and (50), but an intermolecular coupling between (47) and (50) failed. This approach was not developed further as intermediates for an attempted intramolecular coupling could not be synthesised. Other ring A precursors (49) had also been prepared from Hagemann's ester in low yield and some development was necessary if these were to be incorporated into the synthesis of the A/D component.

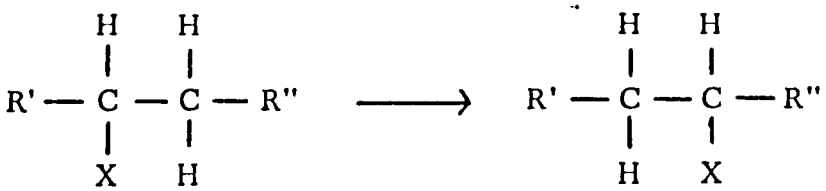
It was at this stage that the Warwick group began collaboration in the work. A new synthesis of ethyl Hagemann's ester (51) and its methyl and t-butyl analogues was developed,[16] enabling a more satisfactory synthesis of (49) and other derivatives. The trans ring geometry of (49) was also proved.[17] For the formation of the 1,19 bond a non-reversible reaction was preferred. Secondary nitronate anions are known[18] to couple with geminal halonitro compounds yielding highly substituted vicinal dinitro compounds (Scheme VII) via a radical-anion mechanism.[19] To use this discovery in the synthesis it was necessary to examine the reaction of primary nitronate anions under similar conditions. Results obtained[20] in this laboratory using model compounds were not encouraging and some further attempts using thallium nitronate salts are discussed in the text. This radical anion coupling reaction using secondary nitronate anions was also successful with simple geminal dinitro compounds.[21] The reaction's limitations with hindered systems and primary nitronate anions have been examined and are described here.

The objective of the following synthetic studies was to apply the coupling reaction of anions with gem halonitro or gem dinitro compounds to the synthesis of the A/D component via the use of precursors (52) and (47). Structural modifications were made so intramolecular couplings could also be attempted, based on preparations from available precursors (Chapters 1 and 2). The possibility of using a Michael reaction involving an intramolecular reaction of the anion of an amido nitro-olefin with an unsaturated residue was also explored (Chapter 3).

Where 'dead end' routes were encountered, the model compounds were used with a view to making a contribution to general synthetic or other nitro chemistry. In particular, the long range deshielding effects of the nitronate anion were recognised in a derivative of (49) and its uses were examined in studies related to conformational analysis (Chapter 4).

The B_{12} Coenzymes

After Barker's[3] discovery of the participation of adeninyl cobamide coenzyme in the enzymatic conversion of glutamate to 3-methylaspartate, numerous other enzymatic reactions dependent on a corrinoid cofactor were also recognised. These may be conveniently divided into two groups. In the first category are molecular rearrangements involving a 1,2-hydrogen transfer. With one exception (ribonucleotide reductase) these reactions may be represented by the following equation :

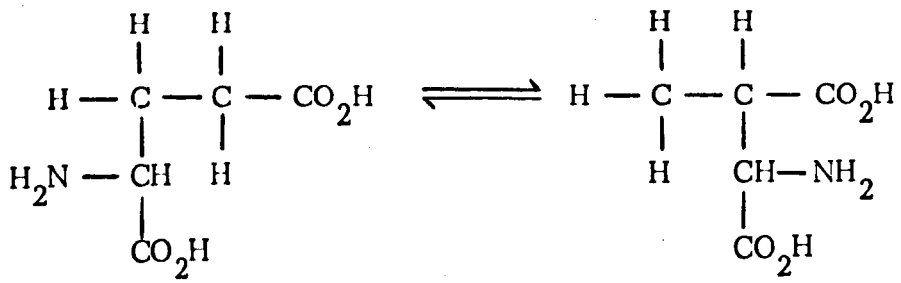


substrate product or intermediate

The enzymatic reactions in this category all require a 5'-deoxyadenosyl corrinoid and are summarised as follows :

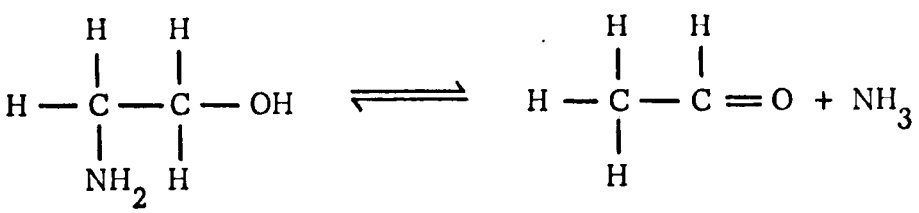
(i) Glutamate mutase

A reversible reaction occurring in Clostridium tetanomorphum



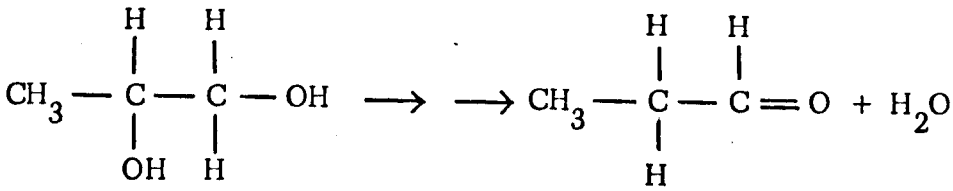
(ii) Ethanolamine deaminase

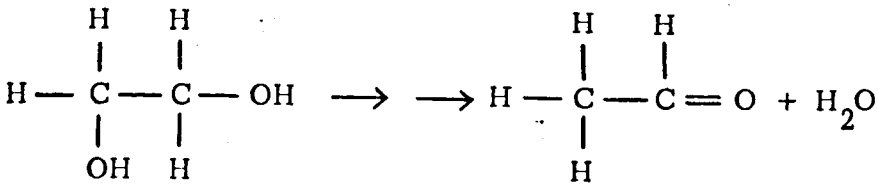
Also from Clostridia



(iii) Dioldehydrase

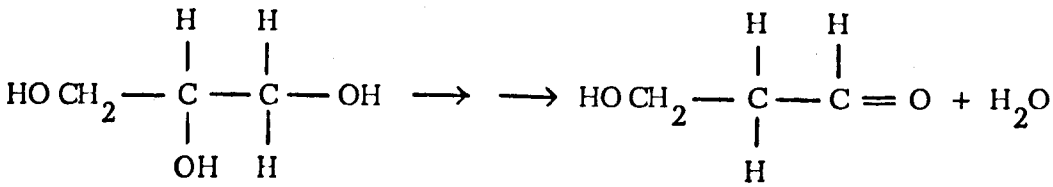
From Aerobacter aerogenes





(iv) Glyceroldehydrase

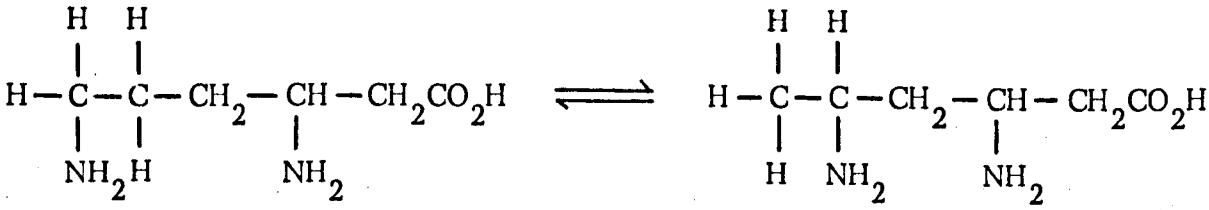
From Aerobacter aerogenes



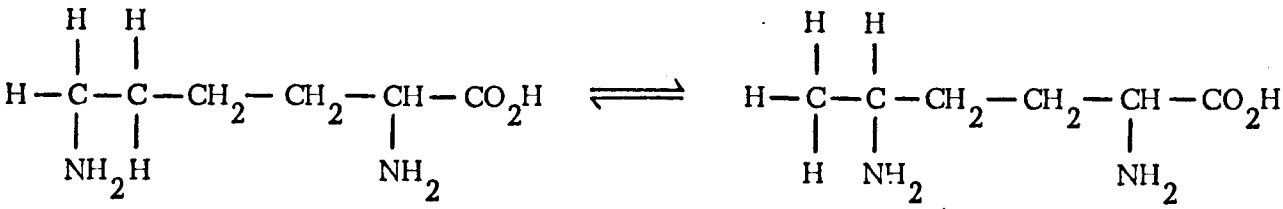
(v) Amino mutases

From extracts of Clostridium sticklandii

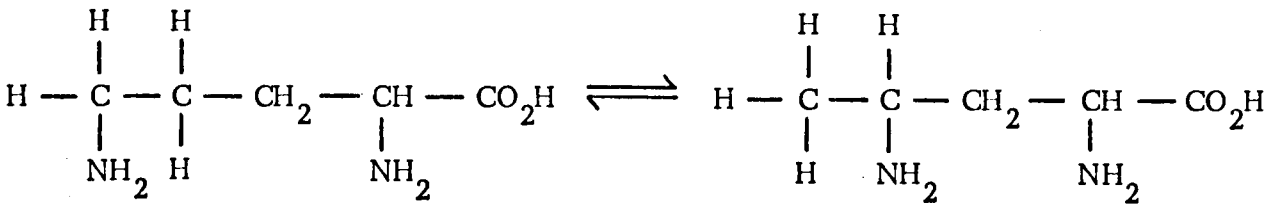
(a) (S) - β - Lysine mutase



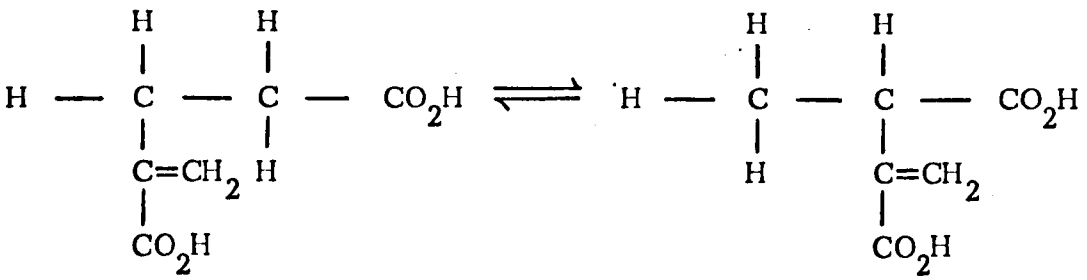
(b) (R) - α - Lysine mutase



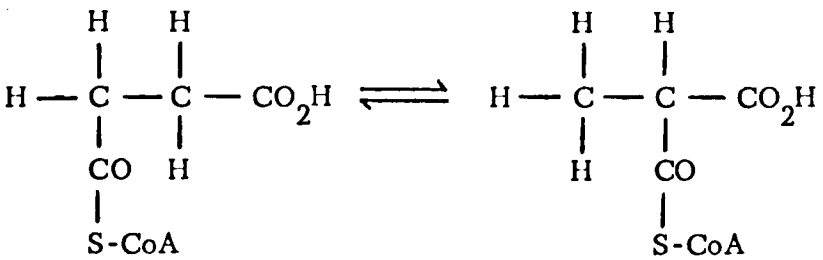
(c) Orthinine mutase



(vi) α-Methyleneglutarate mutase



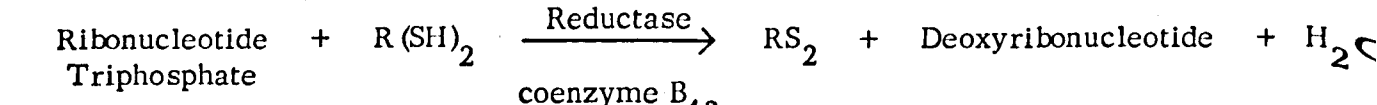
(vii) Methylmalonyl - CoA - mutase



(viii) Ribonucleotide reductase

Occurs in Lactobacillus leichmannii

This type of enzyme catalyses reduction of a ribonucleotide, usually a triphosphate, to the corresponding deoxyribonucleotide using a dithiol as reductant.



Ribonucleotide reductase, although requiring coenzyme B_{12}, and involving hydrogen transfer, differs from the remainder of this category of reactions in that the hydrogen donor and acceptor are different molecules.

The second group of transformations include the methyl transfer reactions catalysed by enzymes which require a methylcorrinoid as the prosthetic group.

(i) Methylation of homocysteine

N^5-methyltetrahydrofolate homocysteine cobalamin methyl transferase is derived from E.coli and animal tissues, and catalyses the transfer of a methyl group from methyltetrahydrofolate to homocysteine forming tetrahydrofolate and methionine. The enzyme also requires adenosyl methionine and a reducing system as cofactors.

(ii) Methane Formation

A corrinoid protein participates in methyl transfer reactions and methane formation in several bacteria including Methanosarcina barkeri and Methanobacterium omelianskii. Extracts from these bacteria form methane from methanol, carbon dioxide, formate, formaldehyde.

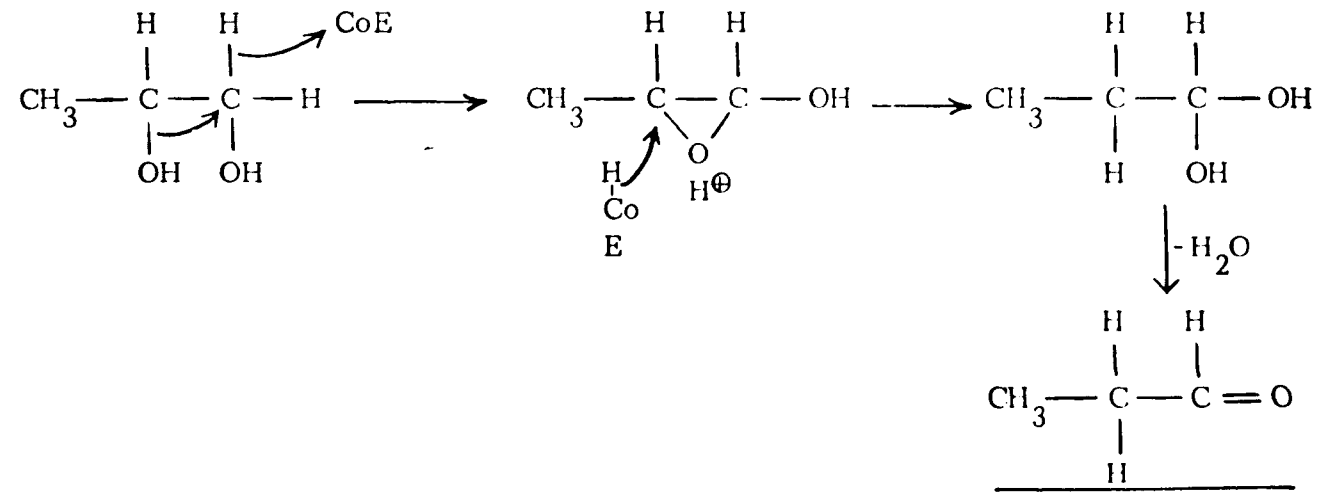
(iii) Acetate synthesis from carbon dioxide

Acetate synthesis in Clostridium thermoaceticum involves firstly, conversion of carbon dioxide to 5-methyltetrahydrofolate. It is implied that the methyl group is then transferred to a corrinoid enzyme and then carboxylation of the methyl-corrinoid to form either an acetyl corrinoid or a carboxy methylcorrinoid occurs. The latter is cleaved to acetic acid, regenerating the corrinoid enzyme. However, as yet, the methyl transfer or the carboxylation steps have not been directly demonstrated in an enzymic system.

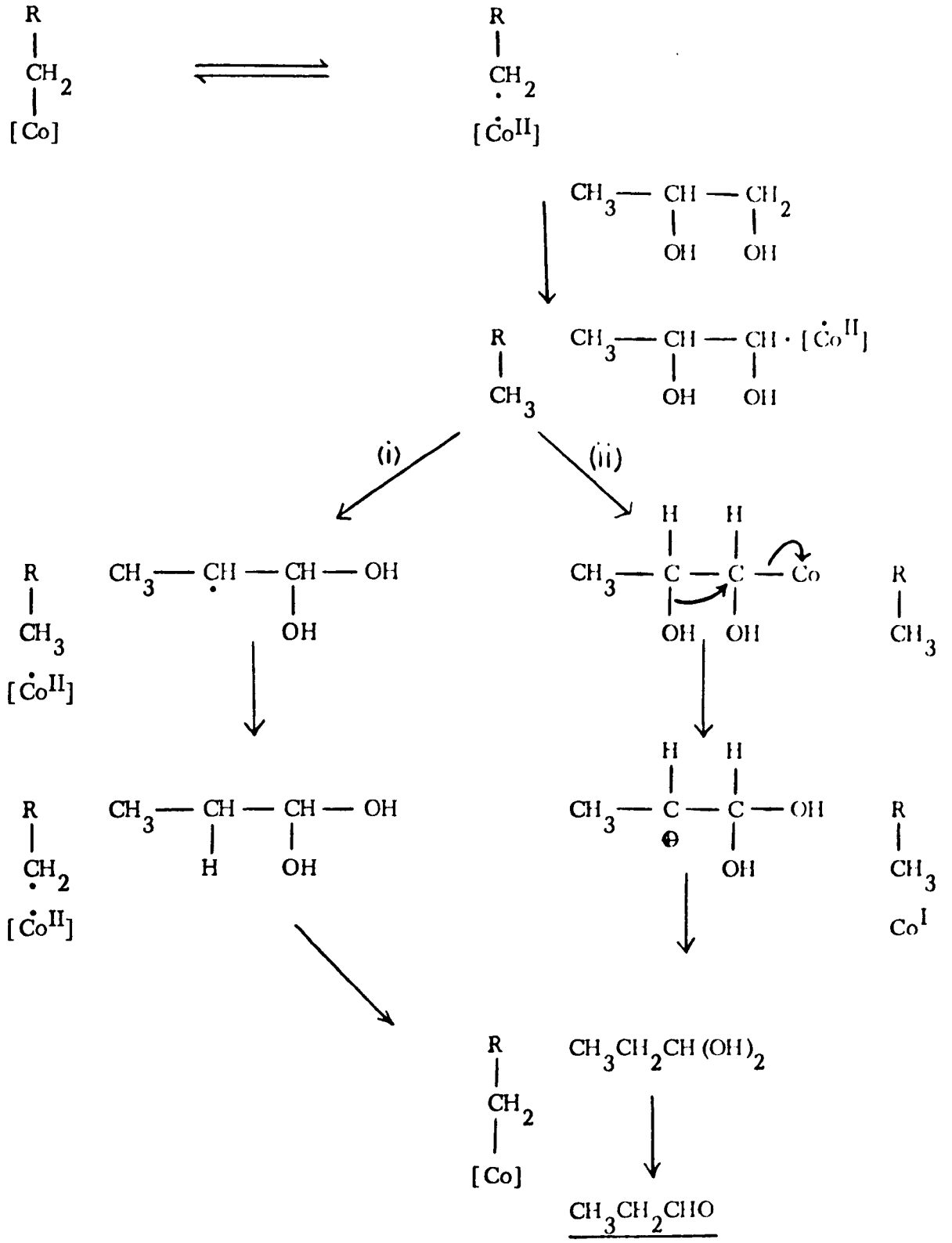
New developments in this area of study have been frequent, as may be judged by the numerous recent reviews[22,23,24] that have appeared on the subject of corrinoid dependent enzymes. Our specific interest is in reactions catalysed by the glyceroldehydrase enzyme formed by Aerobacter aerogenes, strain PZH 572 (Warsaw), so only reactions of this and a similar enzyme, dioldehydrase, are discussed here in any detail.

Dioldehydrase and Glyceroldehydrase

Dioldehydrase, an enzyme extracted from several strains of Aerobacter aerogenes, requires 5'-deoxyadenosylcobalamin as coenzyme for the catalytic conversion of (R) or (S) 1,2-propanediol to propionaldehyde, and 1,2-ethanediol to acetaldehyde. Glyceroldehydrase is an enzyme closely related to dioldehydrase and is also extracted from certain strains of A.aerogenes. In addition to the re-



Scheme VIII[28]



Scheme IX[29a]

actions catalysed by dioldehydrase, glyceroldehydrase also converts glycerol to 3-hydroxypropionaldehyde. Evidence has been presented indicating that the mechanism by which these two enzymes operate is very similar.[25]

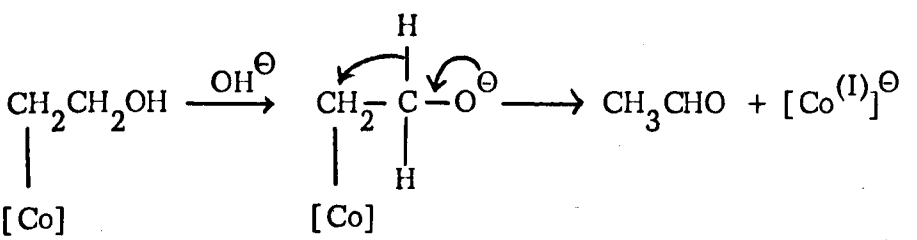
Abeles demonstrated[26] that the C-1 hydrogen of the substrate is transferred to C-5' of the deoxyadenosyl cobalamin, forming 5'-deoxyadenosine as intermediate, in which the methyl group equivalences the one hydrogen atom from the substrate with two of the coenzyme. The C-2 hydrogen of the product is ultimately derived from this methyl group in 5'-deoxyadenosine. The enzymatic conversion of stereospecifically labelled 1,2-propanediol takes the following course :[27]

$$(R) - CH_3CHOH\ CHDOH \longrightarrow CH_3CHD\ CHO$$
$$(S) - CH_3CHOH\ CHDOH \longrightarrow CH_3CH_2\ CDO$$

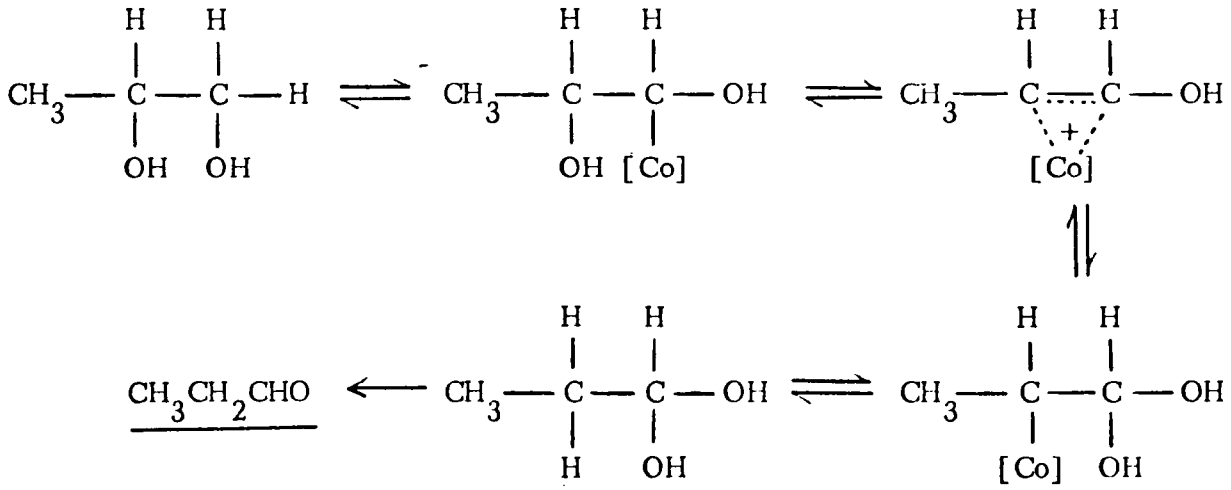
Stereospecific transfer of an oxygen atom from C-2 of the substrate to C-1 of the product (or to water, depending on the configuration of the substrate) also occurs.[28] There is considerable interest concerning the mechanism of the reaction and several theories have been proposed.[26,28,29]

Rétey and Arigoni[28] originally suggested the mechanism outlined in Scheme VIII, but the equation has only formal significance since the intermediate hydroxy epoxide so far has no parallel in organic chemistry.

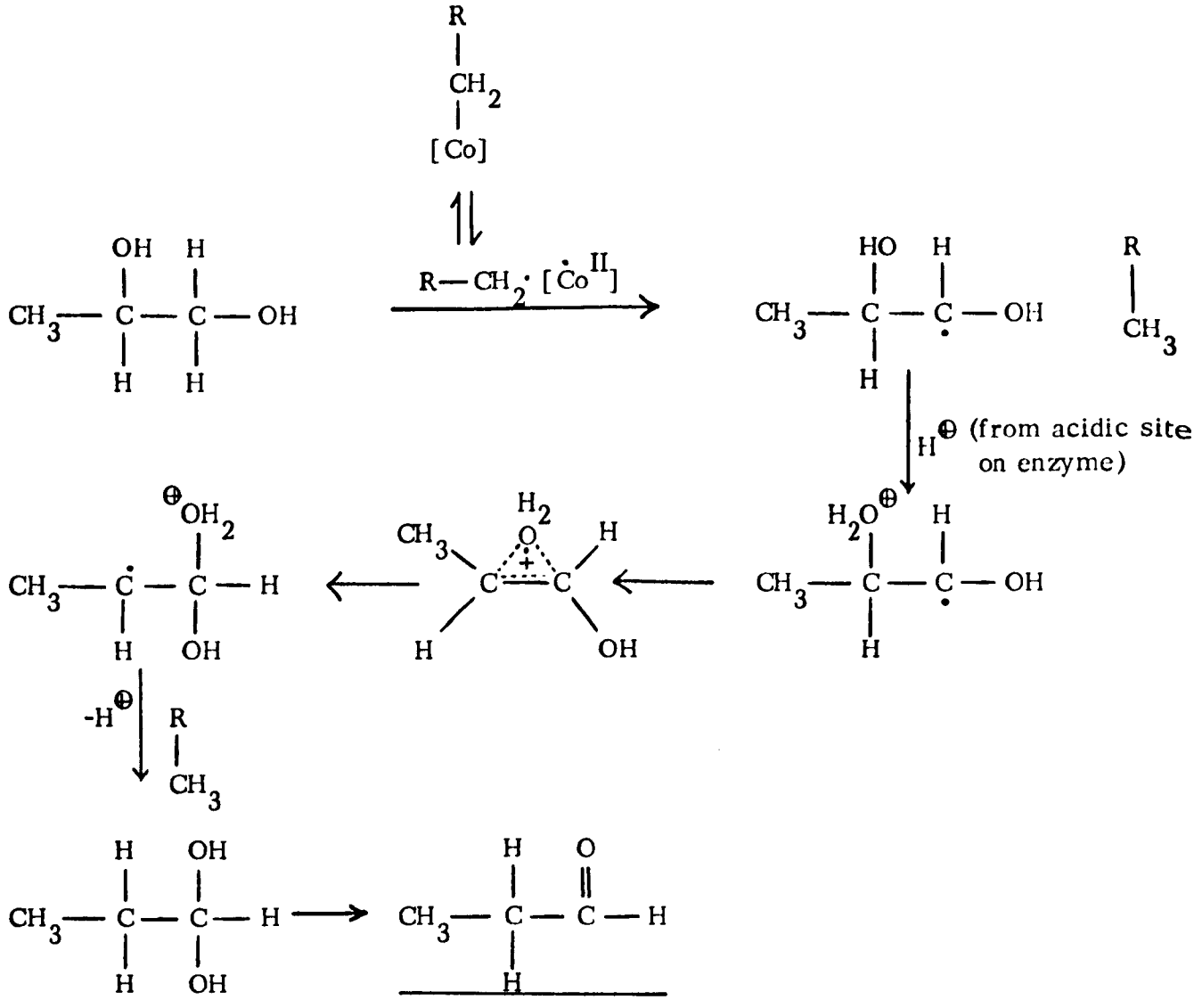
From a study of model reactions of the type :



where [Co] is either cobaloxime or cobalamin, Schrauzer[29d] postulated that the above reaction involved a 1,2-hydrogen shift following abstraction of the hydroxyl proton. Applying these findings to the dioldehydrase reaction, the proposed mechanism involves an enzyme-catalysed dissociation of 5'-deoxyadenosylcobalamin to the highly reactive cob(I)alamin and enzyme bound 5'-dehydroadenosine. The powerfully nucleophilic cob(I)alamin reacts with the diol displacing a hydroxyl group to form a hydroxyalkylcobalamin. This undergoes conversion to the aldehyde, regenerating the cob(I)alamin as described above. Cob(I)alamin recombines with



Scheme X[29b]



Scheme XI[29c]

dehydroadenosine to regenerate the coenzyme. The hydrogen transfer that is observed in the enzymic reaction is attributed to a side reaction; an exchange of hydrogen between dehydroadenosine and enzyme-bound product.

Recent evidence gained from electron spin resonance studies with diol-dehydrase[30,31] and glyceroldehydrase[29a] have indicated that the rearrangements catalysed by these enzymes involve radical intermediates. Nonetheless, it has not yet been possible to identify all the intermediates in the reaction; either pathway (i) or (ii) in Scheme IX is acceptable based on available experimental evidence.

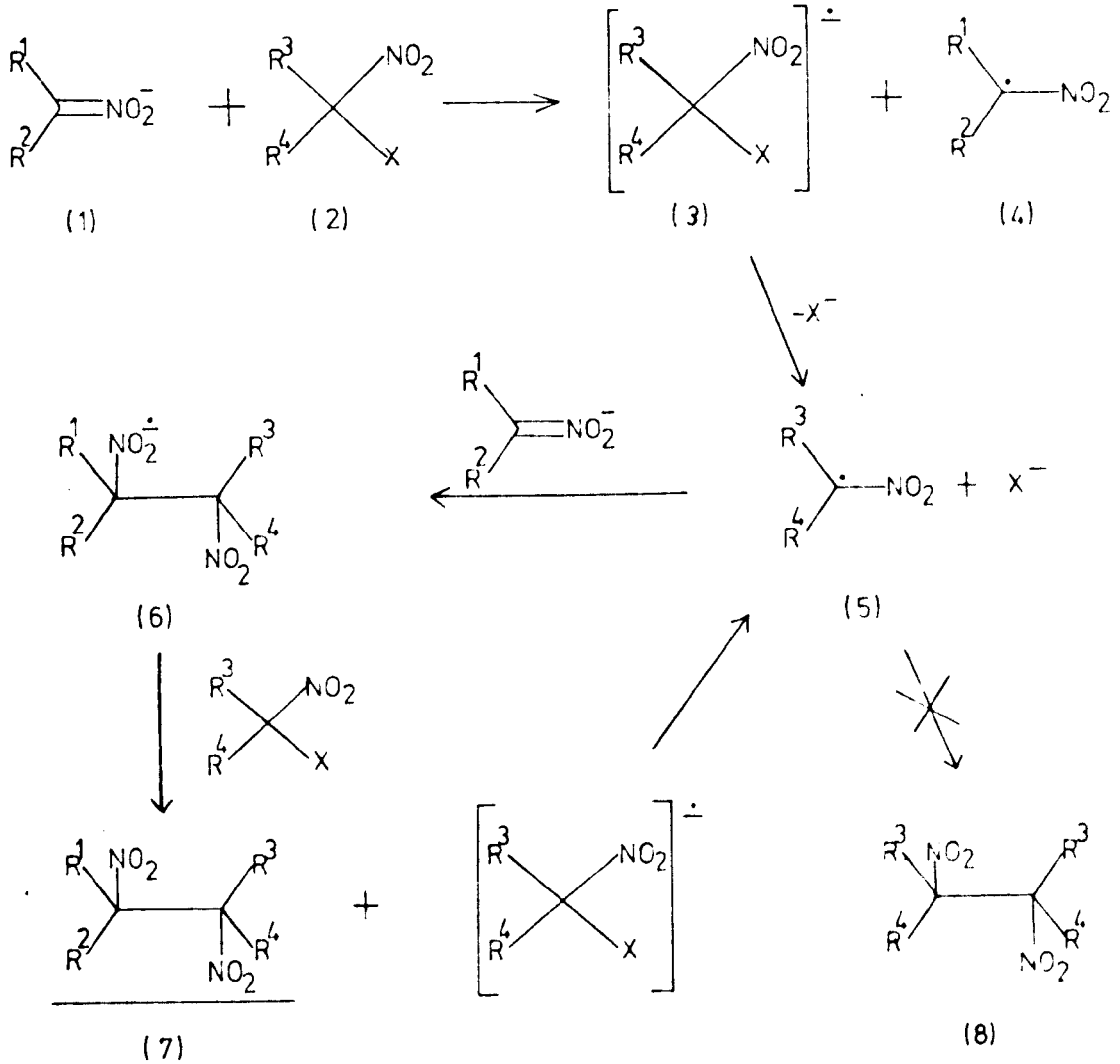
Model reactions using cobaloximes led Babior and Dolphin to propose an intermediate Co^{III} π-complex in the enzymic reaction (Scheme X). Firstly, homolytic fission of the Co - C bond of the coenzyme occurs as proposed above. Then transfer of the migrating hydrogen from C-1 of the substrate to the C-5' of the coenzyme-enzyme complex, concomitant with binding of the substrate at C-1 to the cobalt atom gives a σ-complex. Enzyme assisted removal of the hydroxyl group at C-2 of the substrate leads to the formation of the Co^{III} π-complex. Re-addition of hydroxyl at C-1 followed by breaking of the cobalt to substrate bond, and then remaking of the cobalt-carbon bond in the coenzyme completes the cycle.

A more theoretical approach has come from Golding and Radom.[29c] Ab initio calculations indicate that protonation of the migrating group in 1,2-intramolecular transfer reactions in simple systems, such as the hydroxyethyl radical, promotes such a rearrangement. Calculations also suggest that an intramolecular 1,2-shift of a hydroxy group to a radical centre (as in pathway (i), Scheme IX) is unlikely. Applying these theories to reactions catalysed by 5'-deoxyadenosyl cobalamin, yet another alternative mechanism arises (Scheme XI), consistent with experimental results so far obtained in other studies.

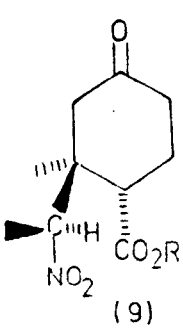
The continued interest in this area has prompted groups to develop techniques to attempt to gain more information about intermediates in these corrinoid dependent enzymatic reactions. For example, low temperature electron spin resonance measurements have recently been used[32] for rapid kinetic studies on the rate of formation of radicals in the reaction of dioldehydrase with propanediol. Fluorine is a particularly useful element to incorporate into a substrate as, of

course, the ^{19}F n.m.r. spectrum of intermediates can be examined. The objective of our study of reactions catalysed by glyceroldehydrase was to synthesise substrates which might be potentially useful in yielding more information pertaining to the mechanism of the rearrangement. 3,3,3-Trifluoro-1,2-propanediol was chosen as a suitable model to examine as there was a reasonable likelihood of it acting as a substrate, and also because it offered the advantages of ^{19}F n.m.r. The synthesis of this compound and its reactions with glyceroldehydrase (from A.aerogenes PZH 572) are recorded in Chapter 5. Several other halogenated diols were synthesised and a preliminary examination of their reactions with glyceroldehydrase was made.

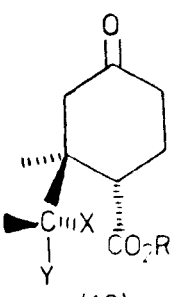
CHAPTER I



SCHEME I



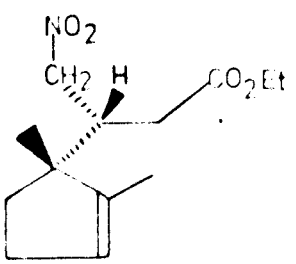
(9)



(10)

X = Halogen

Y = NO_2 , NO



(11)

Halogenonitro and Halogenonitroso Compounds

When I began studies on the synthetic approaches to vitamin B_{12}, a reaction of some promise for forming the bond ultimately to become C1,19 in the corrin nucleus was that between a nitronate anion and a gem halogenonitro compound. This photochemically assisted radical-anion reaction[19] is irreversible and a proposed[19] mechanism is shown in Scheme I. This idea was particularly attractive as it would simultaneously introduce nitrogen functionalities at both carbons 1 and 19. Ring A intermediate (9) and ring D intermediate (11) were already available as possible precursors to the required A/D component. As the nitronate anion would be derived from the primary nitro compound (11), and as the previous studies[19] had employed only secondary nitronate anions, it was first necessary to determine whether the reaction between a primary nitronate anion and a geminal halogenonitro compound is synthetically useful. Bromonitro compounds related to (10) had been already prepared by Dr. A. L. Begbie[17] and some initial coupling studies involving model compounds were carried out by Dr. W. R. Bowman.[20] Some of their work is discussed in this chapter of necessity and for completeness. The intramolecular reaction of a diethylamidomalonyl anion with a bromonitro centre was also examined using a B_{12} ring A precursor. Furthermore, the reaction of primary nitronate anions with gem halogenonitroso compounds was studied as an alternative possibility for coupling.

Coupling Studies in Model Bromonitro Compounds*

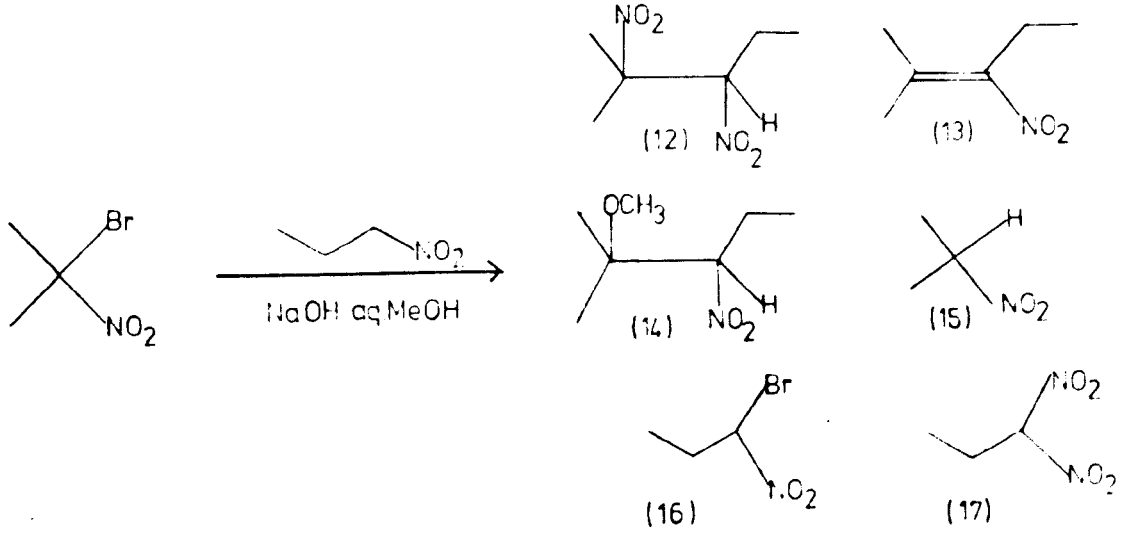
Preliminary studies[20] showed that the reaction between 2,2-bromonitropropane and 1-nitropropane in 85% methanol-water containing two equivalents of sodium hydroxide, afforded a complex mixture of products (Scheme II). Coupling occurred to give the unsymmetrical vicinal dinitro compound, 2-methyl-2,3-dinitropentane (12) in low yield. However, this compound had partly eliminated HNO_2 forming the nitro-olefin, 2-methyl-3-nitropent-2-ene (13) which, in turn, reacted with methanol giving the methoxy adduct (14). Side reactions involving bromine and nitrogroup exchange led to products (15), (16) and (17). Further development of the coupling reaction eliminated the addition of solvent to the nitro-olefin, but, in general, the product mixture contained starting materials,

*N.B. All attempted coupling reactions between nitronate anions and bromonitro compounds were irradiated with white light.

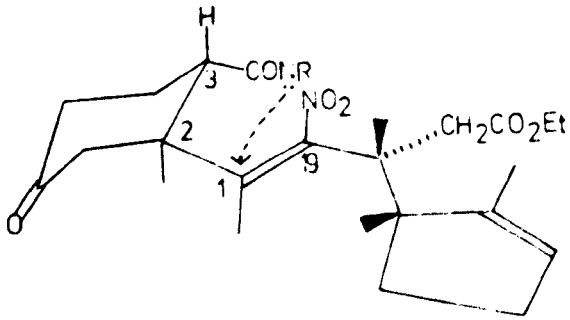
together with (12) and (13), the ratios of which were dependent on the duration of the reaction. It was felt that nitro-olefin formation was not necessarily undesirable should a coupling be successful with B_{12} precursors. If coupling of (10) and (11) led predominantly to a nitro-olefin, it was possible that this could be developed further by, say, incorporating an amide function at C-3. This would offer the possibility of intramolecular cyclisation to reintroduce the nitrogen functionality at C-1.

Consequently, some attempts were made to develop conditions for a bromonitro coupling reaction whereby either of the model compounds (12) or (13) could be obtained in pure form. Current interest in thallium compounds in organic synthesis[33] prompted an intensive examination into uses of thalliumnitronate salts. Thallium (I)ethoxide forms crystalline, stable sharp melting salts with a wide range of acidic substrates (pKa < 18). These salts react with electrophilic reagents and their special utility in organic synthesis is a consequence of the regiospecificity observed in such reactions. For example, 1,3-dicarbonyl compounds are converted quantitatively to thallium (I) salts in hexane, and these may be alkylated by alkyl halides regiospecifically on carbon.[33,34] Thallium-1-propane nitronate (TPN) was readily prepared by reacting thallium (I)ethoxide with 1-nitropropane in dry tetrahydrofuran. The salt was very insoluble in apolar or aprotic dipolar solvents so all the reactions examined were heterogeneous.

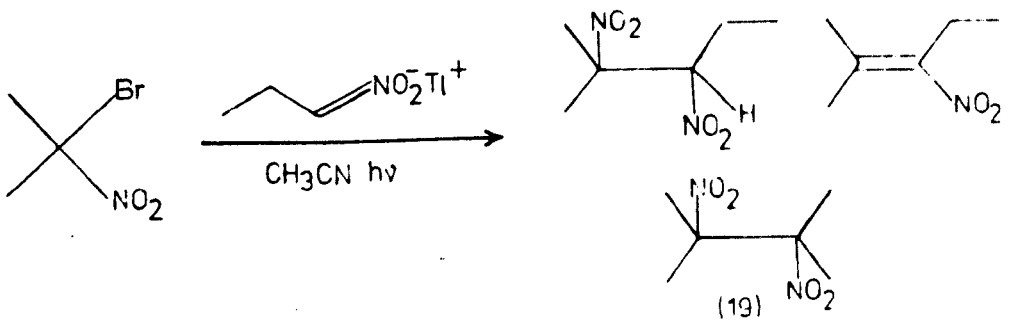
Reaction of TPN with 2,2-bromonitropropane initially formed the vicinal dinitro compound (12) which was converted in time to the nitro-olefin (13); the symmetrical vicinal dinitro compound, 2,3-dimethyl-2,3-dinitrobutane (19), was also a product. Irradiation with white light helped initiation of the reaction, but no reaction occurred unless the temperature was raised above about 40° C. It was thought that a radical mechanism might be involved; the slowness of the reaction, once started, might be attributed to one of the initial products acting as a chain inhibitor. The heterogeneous nature of the reaction could also account for its slowness. Carrying out the reaction at elevated temperatures (60° C) led to a greater proportion of the nitro-olefin. Similar results were obtained using either acetonitrile or tetrahydrofuran as the solvent. The possibility of terminating the reaction when either the vicinal dinitro compound (12) or the nitro-olefin (13) predominated, by use of controlled conditions, was examined. However, a successful

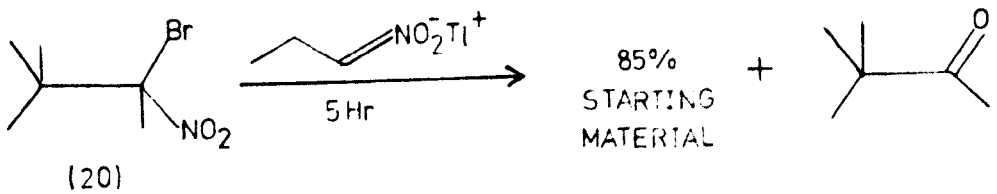


SCHEME II



(18)

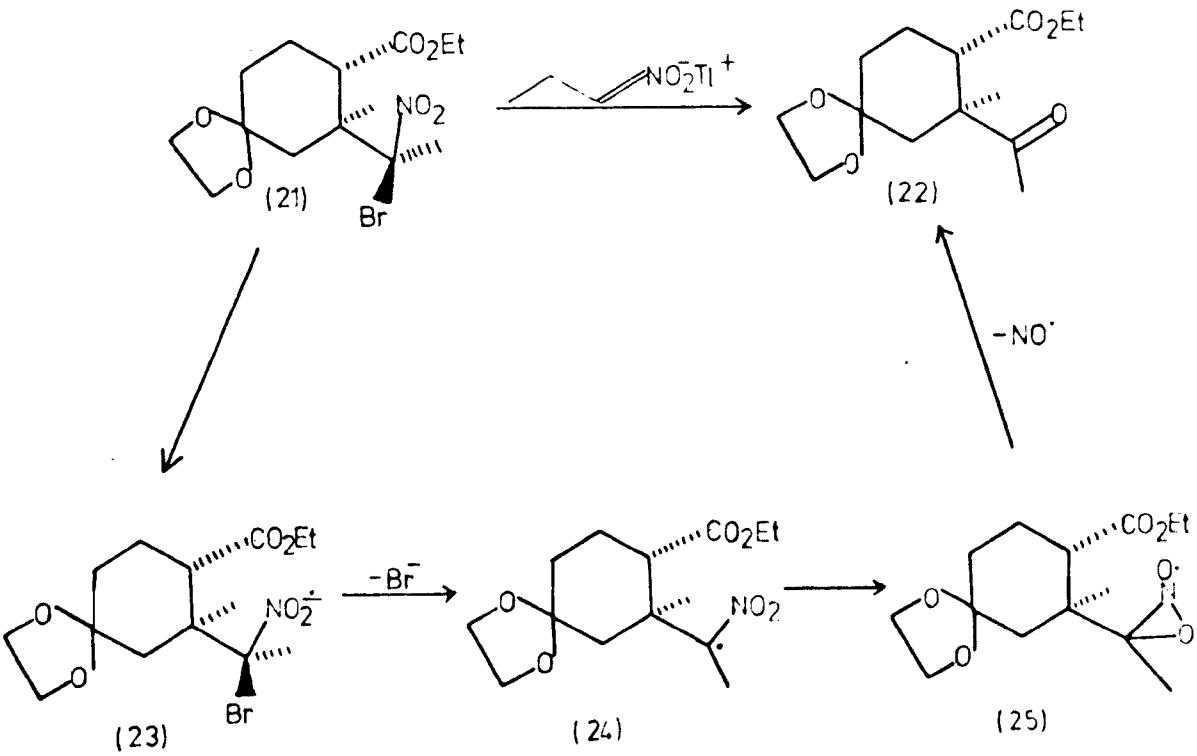


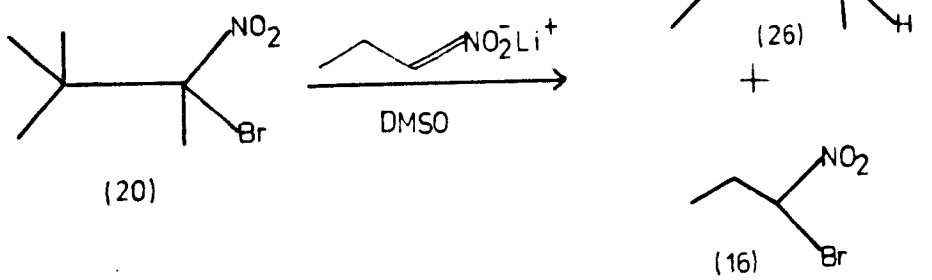


(20)

separation of these two compounds was not achieved, employing a variety of thin layer chromatographic conditions. The vicinal dinitro compound (12) was not isolable by preparative gas chromatography, as some conversion to the nitro-olefin occurred on the column or in the hot injection port. It was evident that a preparation of pure vicinal dinitro compound (12) was unlikely to be successful by this method, although there was some promise for the nitro-olefin (13).

The limitations imposed on the above reaction by steric factors were examined using 2,2-bromonitro-3,3-dimethylbutane (20) as a suitably hindered model. The bromonitro compound was reacted with TPN in acetonitrile under nitrogen and irradiating with white light. Removal of aliquots at intervals and examination of their infrared spectra indicated that a carbonyl-containing compound (ν_{max} 1705 cm^{-1}) was very slowly being produced. It was identified as pinacolone by conversion in situ to its 2,4-dinitrophenylhydrazone and comparison with an authentic sample. It must be emphasised that this conversion of the bromonitro compound to the ketone was extremely slow, as after 5 hours reaction, 85% of the bromonitro starting material could be recovered. A separate experiment established that TPN did not react with pinacolone under similar conditions. The bromonitro ring A precursor (21) reacted with TPN, forming the ketone (22) and other decomposition products.[35] Intermolecular coupling between ring A and ring D precursors using a ring D thallium nitronate salt and a bromonitro ring A intermediate seemed an unlikely event on the basis of this evidence. Also ring A precursor (10) (R = Et, X = Br, Y = NO_2) had previously failed to couple with 1-nitropropane in aqueous methanolic sodium hydroxide, and the products (16) and (17), arising due to bromine and nitrogroup transfers, were identified. The course of these reactions is probably solvent dependent; but the halogen transfer implies that this reaction is faster than transfer of an electron from the nitronate anion to an antibonding orbital of the bromonitro compound. The electron transfer gives a radical-anion and a radical, analogous to species (3) and (4) in Scheme I, respectively. Norman has obtained e.s.r. evidence[36] indicating that radicals such as (5) (Scheme I), rather than self-coupling to (8), preferentially react with a nitronate anion to give the radical-anion (6). A possible mechanism by which a ketone can arise from the bromonitro compound necessitates the formation of a radical-anion such as (3). In hindered systems such as (21), the initially formed


CO2Et
NO2
Br
(21)
NO2⁻Tl⁺
(22)
-NO·
-Br⁻
(23)
(24)
(25)

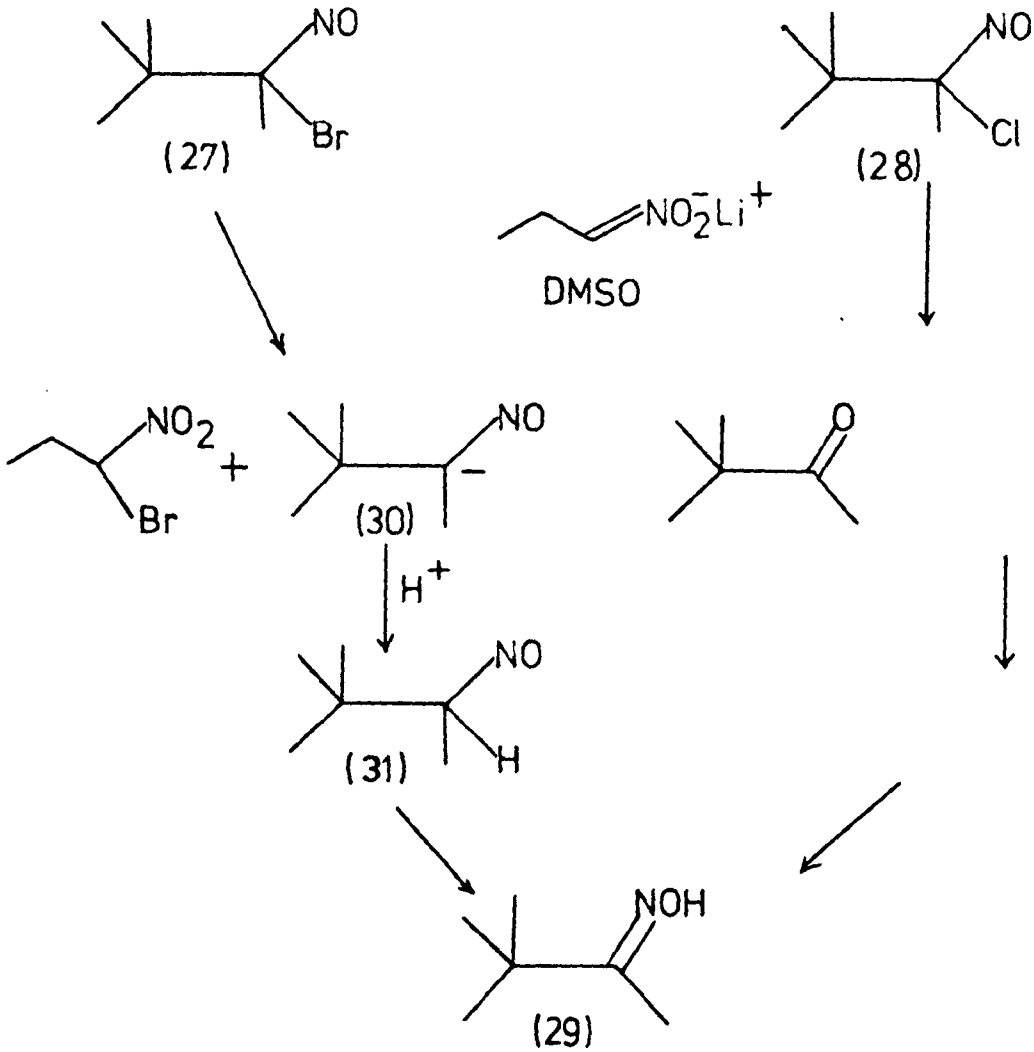

NO2
Br
(20)
NO2⁻Li⁺
DMSO
(26)
+
(16)

radical-anion (23) may lose bromide ion, giving the radical (24). It is proposed that, due to non-bonded interactions, the radical (24) fails to react with the nitronate anion to form a coupled radical-anion analogous to (6) in Scheme I. It is then possible that this radical (24) rearranges to the cyclic intermediate (25) which eliminates NO to give the ketone (22). De Boer has presented e.s.r. evidence[37] indicating that radicals of type (5) form during the photochemical decomposition of geminal nitronitroso alkanes (pseudonitroles) to ketones. Homolytic fission of the C - NO bond likely occurs, generating the radical (5) which is stable for at least 30 minutes at -40° C when derived from 2,2-nitronitrosopropane. A suggested[37] route to ketone involves readdition of the liberated NO to unreacted pseudonitrole generating radical products, which either rearrange directly or first react with radical (5) and then rearrange to ketone. Since the mechanism of ketone formation from bromonitro compounds cannot involve NO, their formation by rearrangement of hindered radicals of type (24) is a reasonable proposal.

In a reaction between lithium 1-propane nitronate and 2,2-bromonitro-3,3-dimethylbutane in DMSO, a major pathway was bromine transfer as 1,1-bromonitropropane (16) and 2-nitro-3,3-dimethylbutane (26) were identified in the mixture of products. This strongly suggests halogen transfer to be faster than formation of a radical-anion in this case. Note that coupling can only take place via the latter species. The synthetic limitations of these reactions were obviously numerous and seemed to preclude successful application to the preparation of an A/D component.

Simple Halogenonitroso Compounds

Although gem halogenonitroso compounds were first prepared[38] at the beginning of this century, there have been few reports concerning their synthetic uses.[39] Hawthorne[40] observed that gem chloronitroso alkanes could be solvolytically converted, with silver ion catalysis using silver perchlorate, to ketones, probably via an α-nitrosocarbocation and derived gem hydroxynitroso intermediate. Since it might be possible to capture this cationic intermediate by a nitronate anion with resultant C - C bond formation, a study of gem halogeno-



SCHEME III

$NO_2^-Li^+$ NO / MeOH	O	NOH	ONO	NO_2 NO_2	NO NO_2
1 Hr	10%	22	7	2-3	2-3
2½ Hr Complete	30-35%	29	12	1-2	0

TABLE I

nitroso compounds was undertaken in this context. Gem halogenonitroso compounds could also react with nitronate anions by an electron transfer mechanism analogous to that described for halogenonitro compounds.

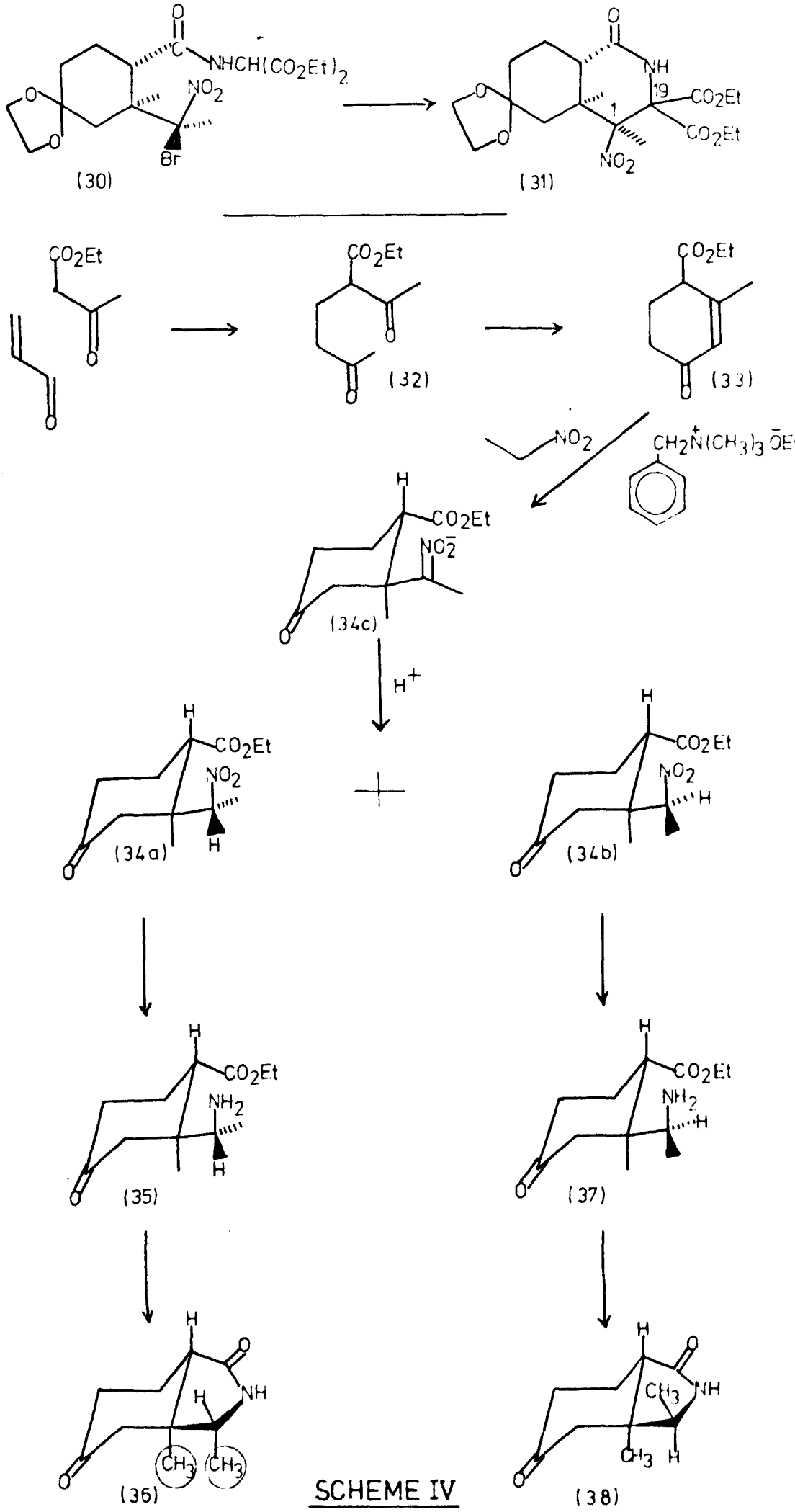
The hindered compounds 2,2-bromonitroso-3,3-dimethylbutane (27) and 2,2-chloronitroso-3,3-dimethylbutane (28) were chosen as convenient models. (27) was synthesised by bromination of pinacolone oxime (29) in aqueous pyridine. Bromination using N-bromosuccinimide (NBS) in aqueous dioxan containing sodium bicarbonate, gave a mixture of bromonitroso and bromonitro compounds. It was later found that oximes can be quantitatively converted to the corresponding bromonitroso compound by NBS in chloroform, with the exclusion of oxygen. The chloronitroso compound (28) was prepared from the corresponding oxime using t-butylhypochlorite as the halogenating agent. This is a general reaction of oximes.[41] Gem halogenonitroso compounds are intensely blue, light sensitive materials, which are stable for long periods at -78° C, excluding oxygen. Provided oxygen and light are excluded they can be safely stored at -20° C for several months. The main contaminant, after storage for any duration, is the corresponding bromonitro compound.

High reactivity of these compounds was observed in several reactions with lithium 1-propanenitronate in DMSO. (All reactions were irradiated with white light to help radical initiation). However, hopes of forming carbon-carbon bonds were unfounded. Bromine transfer was the major reaction using the bromonitroso compound, and pinacolone oxime and 1,1-bromonitropropane were identified as predominant products. Presumably reaction proceeds via (30), which abstracts a proton (probably from water added on work up) to give the nitroso compound (31) which then isomerises to the oxime. A reaction under identical conditions, but using 2,2-chloronitroso-3,3-dimethylbutane was more complex. Addition of the chloronitroso compound to lithium 1-propanenitronate caused the blue colour to rapidly become more intense. Termination of the reaction at this stage by addition of water resulted in only pinacolone oxime being isolated. If reaction was allowed to proceed for a longer period, 2 hours of irradiation with white light were required for the blue colour to fade. The product then was a very complex mixture, and the components could not be identified. Following the reaction by n.m.r. in $^{2}H_{6}$-DMSO indicated that pinacolone was a product of the reaction. No 1,1-chloro-

nitropropane was detected in the mixture. The reactions were undoubtedly complicated by the photochemical conditions that were used.

Gem halogenonitroso compounds are predominantly monomeric in solution; the long wavelength absorption band at about 650 nm is due to an $n-\pi^*$ transition of the nitrosogroup, responsible for the intense blue colour.[41,42] Irradiation with red light leads to excitation of the nitrosogroup and it is highly probable that the excited gem chloronitroso compound loses NO[44,45,46] under the conditions used for attempted coupling reactions. The initial photochemical decomposition of gem chloronitroso compounds always takes place via C-N fission,[44,45] and this is in agreement with calculations [D (C-NO)[46] = 34 - 41 Kcal mole^{-1}, D (C - Cl)[47] = 76 - 80 Kcal mole^{-1}]. The generated NO may react with unreacted chloronitroso compound or the nitronate anion. It was earlier demonstrated[48] that lithium 2-propanenitronate reacted with NO in methanol giving a variety of products (Table 1), principally acetone and acetoxime, after all starting material was consumed.

Some preliminary studies were made to quantitatively remove the halogen from a halogenonitroso compound. The intent was to generate an α-nitroso-carbocation (cf. page 18) probably a highly electrophilic species. Reaction of bromonitroso-dimethylbutane with silver tetrafluoroborate in benzene rapidly precipitated silver halide but a clean organic product was not observed. Further developments were not made but it is possible that some improvement could be achieved by the use of liquid SO_2 as solvent, since this had been used for reactions of the related α-chloronitrones.[49]


NHCH(CO2Et)2
NO2
Br
(30)
NH
19
1
CO2Et
CO2Et
NO2
(31)
CO2Et
(32)
(33)
NO2
CH2N(CH3)3 OEt
H
CO2Et
NO2
(34c)
H+
(34a)
(34b)
NH2
(35)
(37)
NH
CH3
CH3
(36)
(38)

SCHEME IV

Synthesis of a Ring A Precursor Having Functionalities for Intramolecular Cyclisation of an Anion with a Bromonitro Centre

Concurrent with experiments investigating the limitations of intermolecular coupling reactions between a nitronate anion and halogenonitro compounds, the model (30) was devised to explore an intramolecular approach. This incorporates a bromonitro centre with a diethylamidomalonyl moiety to facilitate anion formation. The latter also provides the required nitrogen atoms in the cyclised product (31). If compound (30) cyclised then it would be modified to incorporate a derivative of ring D precursor (11).

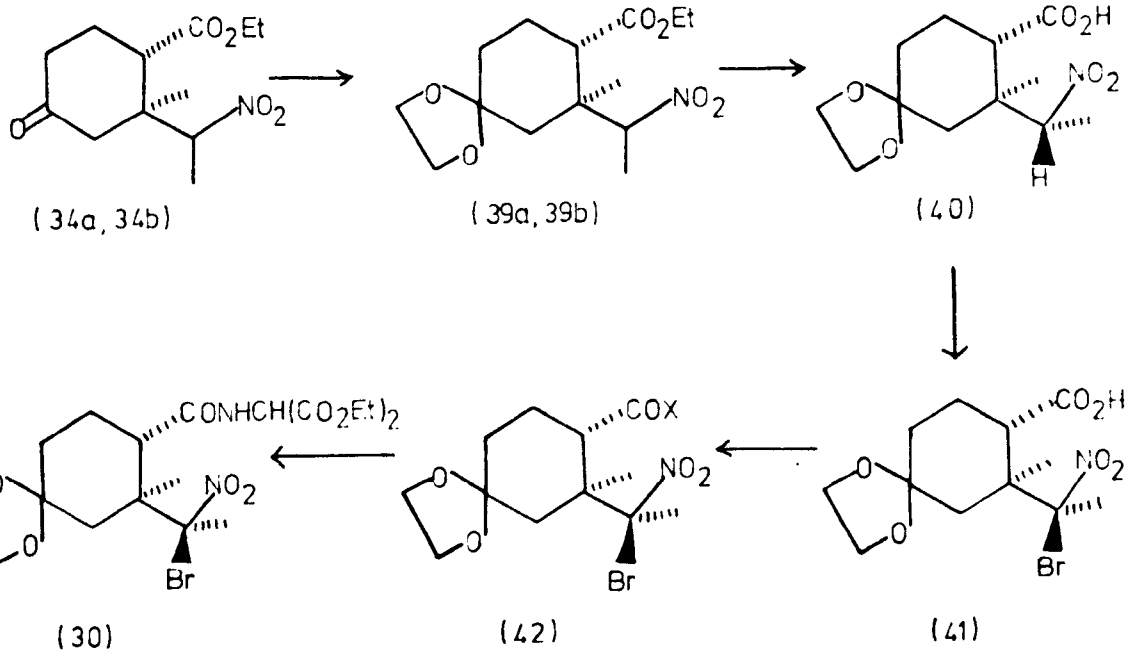
The key 'building block' for all our studies concerning ring A intermediates were the Hagemann's esters (4-alkoxycarbonyl-3-methyl-cyclohex-2-enone) for which a new, convenient systhesis was developed.[16] For the preparation of ethyl Hagemann's ester, base catalysed addition of ethyl acetoacetate to methyl vinyl ketone gave the dioxo ester (32). Catalytic quantities of pyrrolidine and glacial acetic acid effected rapid cyclisation of the dioxo ester to ethyl Hagemann's ester (33).

Nitroethane Adducts of Ethyl Hagemann's Ester

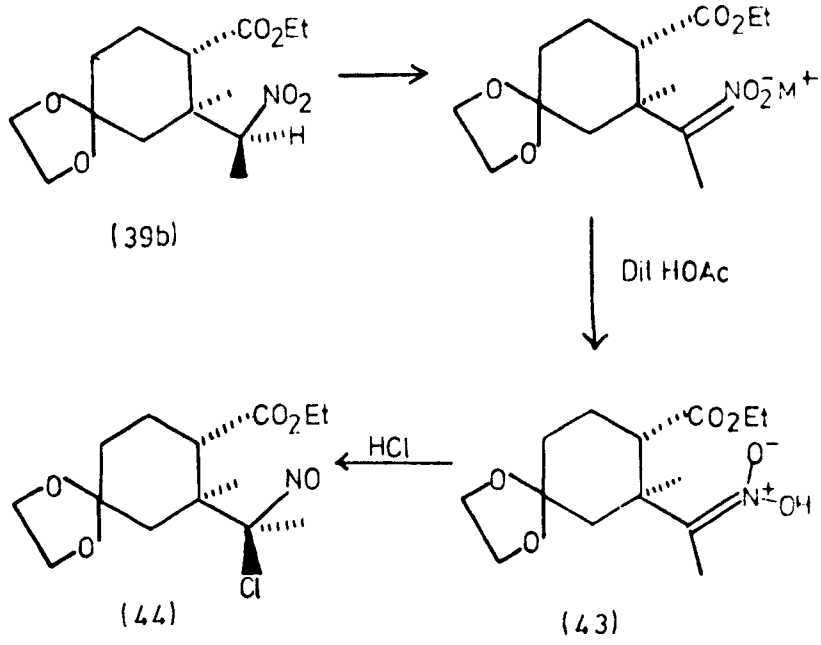
Base catalysed addition of nitroethane to ethyl Hagemann's ester gave a mixture at equilibrium, containing two isomeric nitro-adducts. Of the possible diastereoisomers only those with trans ring stereochemistry were produced ((34a) and (34b)).[17] It was also demonstrated that these isomers were epimeric in the 2-nitroethyl group, since they were related by the same nitronate anion (34c). (See Chapter 4). The relative configuration of each epimer at the nitroethyl centre has yet to be established. Kinetic protonation of the nitronate anion (34c) gave predominantly one liquid isomer (34a), and it was anticipated that protonation should occur from the least hindered side. Molecular models indicated that (34a) should be the product in this case. Base catalysed equilibration of the liquid isomer gave a mixture containing an excess of crystalline epimer (34b). The two epimers were readily distinguished by the chemical shift of the nitro-methine proton in their ^{1}H n.m.r. spectra.

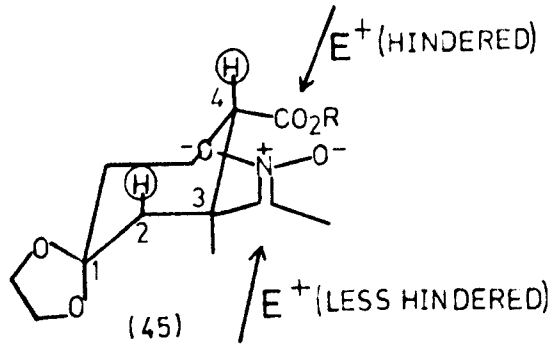
A proposed sequence (Scheme IV) for the resolution of the relative configuration at the 2-nitroethyl group is being investigated by Dr W.R. Bowman.* Both epimers (34a) and (34b) are to be converted to their corresponding isomeric

* At Loughborough University of Technology.


CO2Et
NO2
(34a, 34b)
(39a, 39b)
CO2H
(40)
(41)
CONHCH(CO2Et)2
COX
Br
(30)
(42)

SCHEME V


CO2Et
NO2
H
(39b)
NO2⁻M⁺
Dil HOAc
HCl
NO
Cl
(44)
(43)


E+ (HINDERED)
CO2R
E+ (LESS HINDERED)
(45)

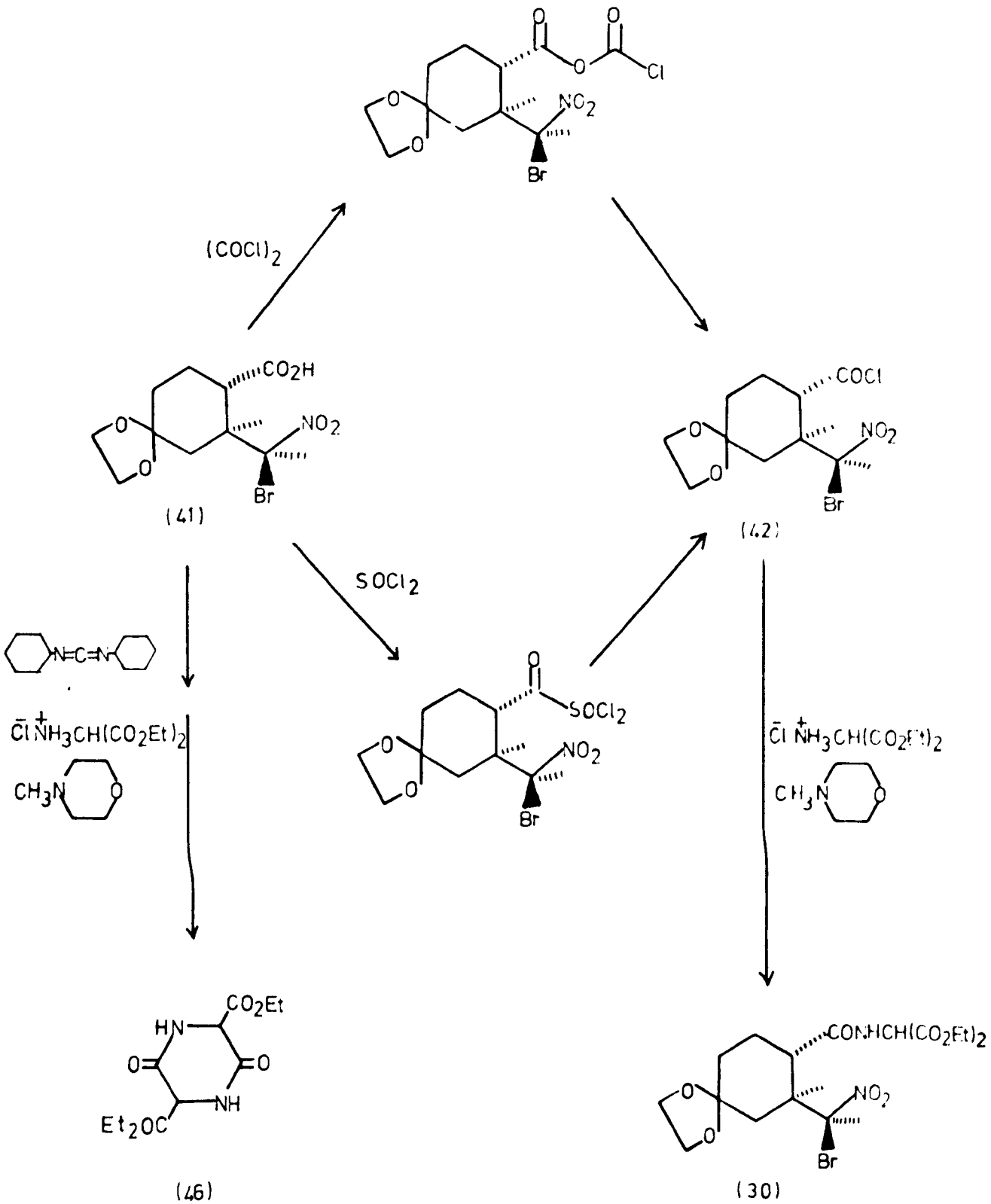
lactams (36) and (38) via the amino compounds (35) and (37) respectively. In lactam (36) the two methyl groups are eclipsed and this interaction should be detectable using the Nuclear Overhauser Effect:[50] irradiation of one methyl group should cause an increase in the intensity of the transition due to another methyl group only in an eclipsed position (i.e. with (36) but not (38)), thus enabling the stereochemistry of the nitro compounds, from which the lactams may be derived, to be assigned.

Synthesis of 4-Carboxamidodiethylmalonyl-3-methyl-3-(2',2'-bromonitroethyl) Cyclohexanone Ethylene Ketal (30)

The planned synthetic route to the bromonitro ring A precursor (30) is outlined in Scheme V.

The liquid and crystalline nitro-esters (34a) and (34b) were quantitatively ketalised to the corresponding liquid and crystalline ketals (39a) and (39b), which differed in configuration only at the nitroethyl group. Aqueous basic hydrolysis of either liquid or crystalline ethylene ketal nitro-esters (39a), (39b) gave the same carboxylic acid (40). Its n.m.r. spectrum and sharp melting point indicated that (40) was a single epimer. Bromination of the anion of (39) also afforded predominantly one epimer.[35]

This simple ester hydrolysis was not completely straightforward: it was slow, and refluxing with a large excess of sodium hydroxide (20 equivalents) was necessary for complete conversion. When a smaller excess (3 equivalents) of base was used, then on neutralisation with acetic acid, a second product was isolated with the carboxylic acid (40). From spectroscopic evidence, and its instability at room temperature, the nitronic acid structure (43) is assigned to this product. (43) could be prepared in high yields by formation of the nitronate anion of (39) in aqueous solution, followed by addition of dilute acetic acid. The weak acid protonated the anion completely on oxygen. No hydrolysis of the ethoxycarbonyl group occurred under the basic conditions. The u.v. spectrum of (43) showed a strong $\pi - \pi^*$ absorption maximum at 229 nm (ϵ, 7,800); typical values for nitronic acids in ethanol or water are within the range 220 - 230 nm ($\epsilon \sim 10,000$).[51] The i.r. spectrum showed no absorption due to N=O stretching. Treatment of (43) with chloroform containing hydrogen chloride restored this absorption in the i.r. spectrum.The blue oil which resulted is assigned the


(COCl)2
CO2H
NO2
Br
(41)
COCl
(42)
SOCl2
SOCl2
Cl NH3CH(CO2Et)2
CH3N
O
CO2Et
HN
NH
Et2OC
(46)
CONHCH(CO2Et)2
(30)

SCHEME VI

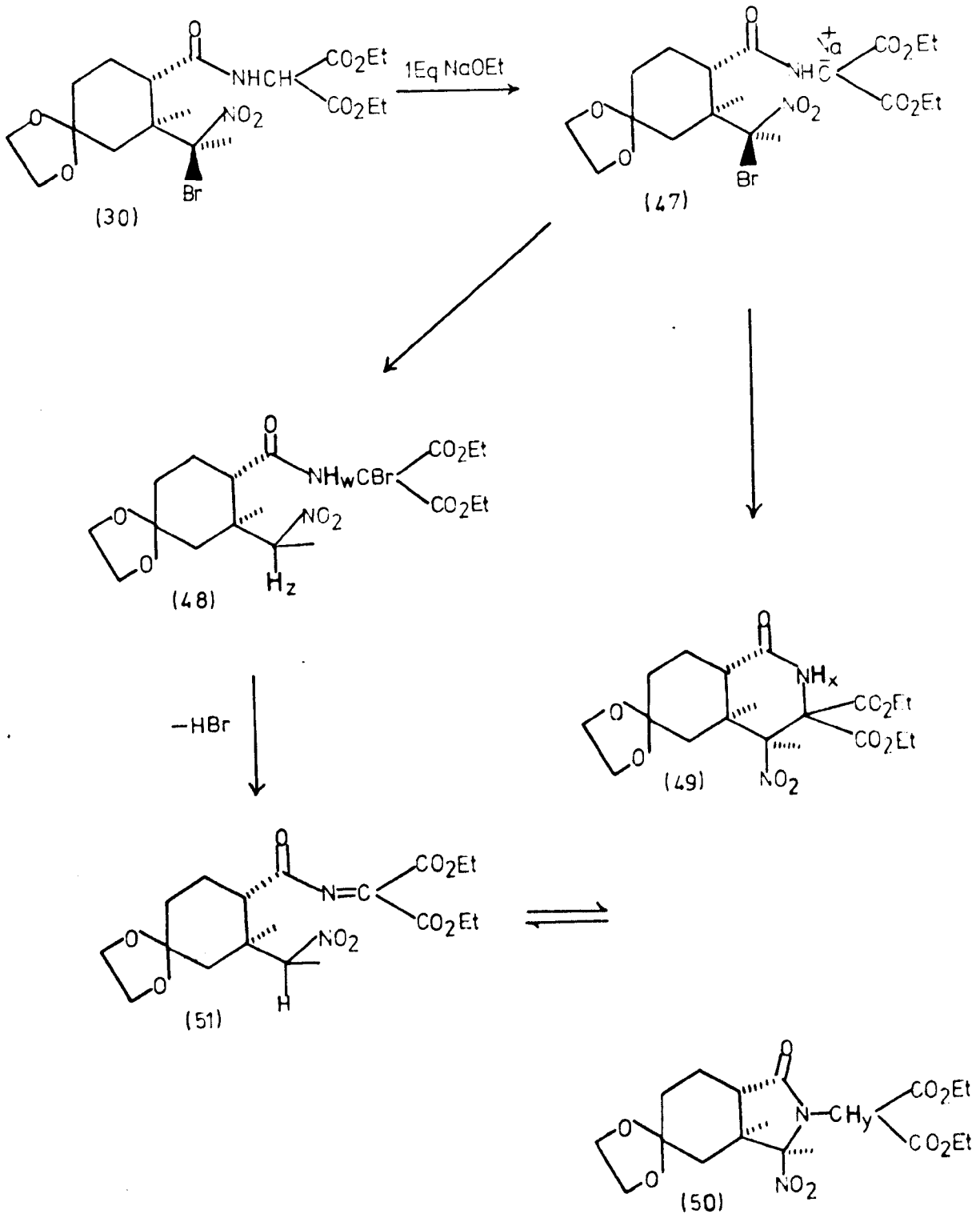
structure of the gem chloronitroso compound (44). As a consequence, the nitronic acid (43) was not without some interest since investigations into model halogenonitroso compounds was a parallel line of study at the time. The nitronic acid route could be convenient for the introduction of the gem halogenonitroso functionality into ring A precursors. However, for coupling purposes, experiments with model halogenonitroso compounds turned out to be of no preparative value, so this area of study was not developed further.

Bromination of the ketal nitro acid (40), *via* its dianion in t-butanol, gave the ketal bromonitro acid (41), again largely as one epimer (cf. bromination of (39) *via* its nitronate anion). These results and those gained from kinetic protonations of nitronate anions,[17] strongly support the suggestion that, in compounds of the type (45), one face of the planar nitronate grouping is considerably more hindered than the other. For this to be so, it is necessary that a conformation be significantly populated, whereby the substituent at C-4 hinders the approach to the nitronate anion. The conformation shown in (45) satisfies this requirement. ^{1}H n.m.r. studies on anions such as that derived from (39) support this interpretation since the axial protons at C-2 and C-4 are markedly deshielded by the nitronate grouping. In contrast, the methyl protons at C-3 are unaffected (see Chapter 4).

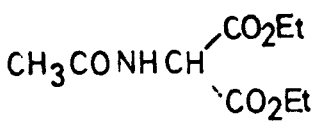
Amide Formation (Scheme VI)

The dicyclohexylcarbodiimide (DCC) method was unsuccessful for activating the ketal bromonitro acid (41). Addition of diethylaminomalonate to a mixture of (41) and DCC resulted only in the formation of the dimer (46) of diethylaminomalonate. Either the initial DCC intermediate was particularly unreactive or it may have reacted with itself *via* transacylation. This behaviour is typical of hindered carboxylic acids.

The acid chloride route to the amide was an obvious alternative. Oxalyl chloride converted the acid (41) to its acid chloride (42), although the i.r. spectrum of this product had a very broad absorption in the carbonyl region, suggesting the presence of several components. This crude acid chloride reacted with diethylaminomalonate, in the presence of N-methylmorpholine, giving a low yield of the required ketal bromonitro amide (30). The product was contaminated with several byproducts, and required repeated recrystallisation to effect their removal.



SCHEME VII



(52)

It was felt that the source of these contaminants may have been in the acid chloride stage. Several methods of preparing the acid chloride were examined. It was found that the ketal bromonitro acid could be converted quantitatively to its acid chloride by treating a suspension of the acid in benzene with a large excess of thionyl chloride. The acid chloride subsequently reacted cleanly with diethylaminomalonate in dichloromethane, affording the bromonitro amide (30) in good yield.

The Cyclisation of (30)

Preliminary studies investigating the reactions of (30) under basic conditions suggested that these might be complex. In methanol, methoxide ion catalysed ester exchange, so deprotonations in alcoholic solvent were performed using sodium ethoxide in ethanol. Reaction of (30) with one equivalent of sodium ethoxide resulted in the formation of an amorphous solid which was a mixture of at least two compounds. A reaction in DMSO using n-butyl lithium as the base gave a product with a similar n.m.r. spectrum, but in this case the material was an oil which failed to crystallise. Purification of the components proved to be particularly difficult. Recrystallisation was not successful and t.l.c. using a variety of conditions did not effect a satisfactory separation. It is possible that the components could be resolved by high pressure liquid chromatography, but we did not have available the necessary equipment.

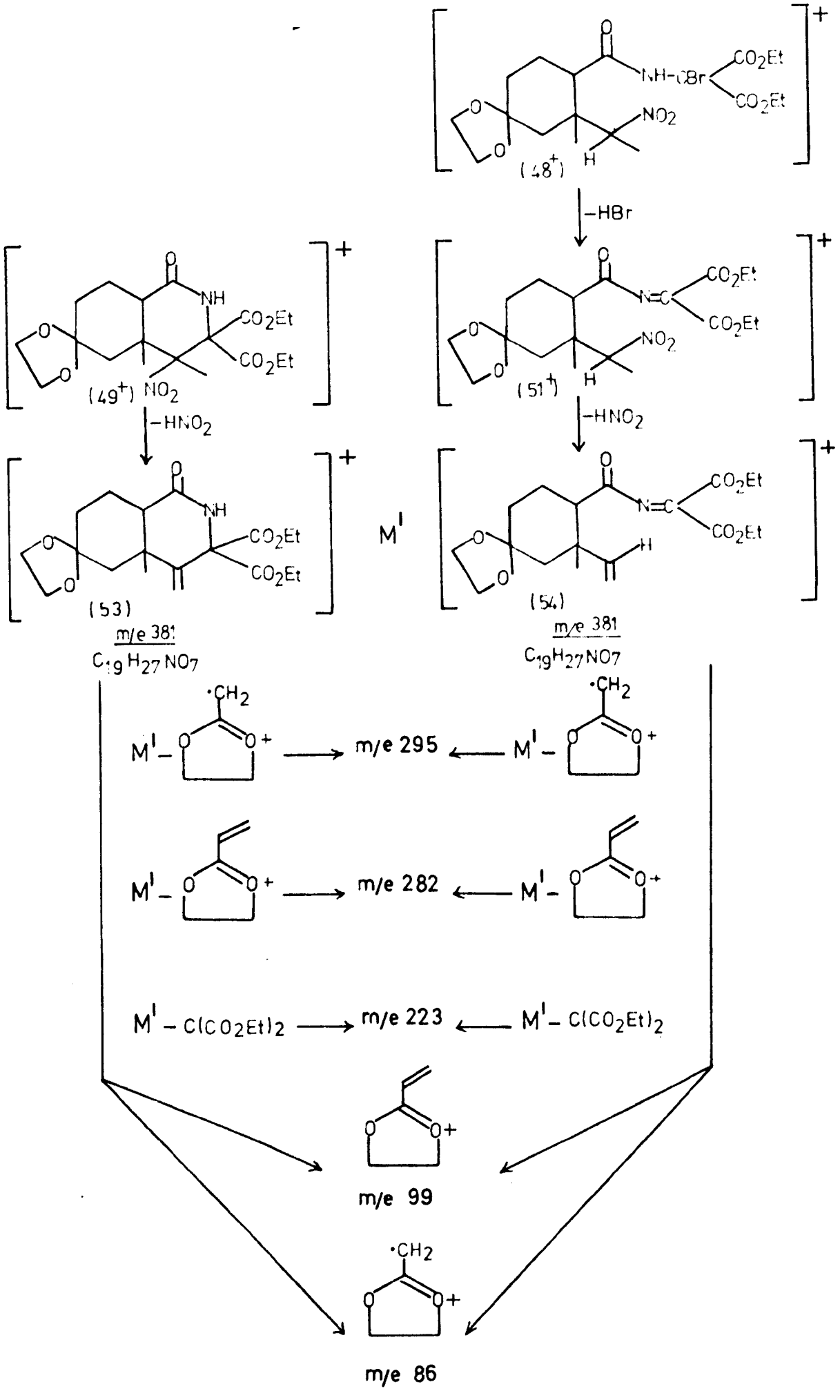
The most probable products derived from reaction of sodium ethoxide with (30) are shown in Scheme VII. Compound (49) was required if further development towards the A/D component was to be successful using this approach. Using strong bases for the deprotonation step, it was possible that cyclisation might occur *via* the amide nitrogen, resulting in compound (50). Spectroscopic evidence could not unequivocally distinguish between these two compounds. Compound (48) is a product which could have arisen by bromine transfer, a reaction we observed when primary nitronate anions reacted with hindered bromonitro compounds (cf. page 18). Van Tamelen showed[52] that reaction of 2,2-bromonitropropane with sodium malonic ester in aqueous solution did not lead to normal alkylation. Bromine exchange occurred first giving bromomalonic ester, which, in the presence of excess sodium malonic ester, reacted to form ethane tetracarboxylic ester.

The differing acidities of compounds (48), (49) and (50) should have enabled a separation of (49) from (48) and (50). However, basification of the mixture with sodium ethoxide and then extraction after dilution with water (pH 9) gave a product whose spectroscopic properties were very similar to the mixture.

The n.m.r. spectrum of the unknown, resulting from reaction of (30) with sodium ethoxide, showed two very broad signals at δ 5.05 and δ 7.25, each representing half of one proton, the higher field signal possibly being due to Hy in (50) and the low field signal due to Hx in (49). In the n.m.r. spectrum of the product derived from the reaction of (30) with n-butyl lithium, the resonance at δ 5.05 resolved more clearly and was possibly a quartet which could be due to Hz in (48). It was difficult to define the methyl doublet which should be coupled to this methine proton, but the presence of a nitromethine proton in the n.m.r. spectrum offers strong evidence for bromine exchange having occurred.

In the i.r. spectrum of the unknown, amide I carbonyl absorption occurred at 1690 cm^{-1}, both in the solid state and in solution. Amide I absorption in (30) was at 1660 cm^{-1} in the solid state and 1680 cm^{-1} in solution. Comparison with simple cyclic amides indicated that similar absorptions in 2-piperidone occur at 1655 cm^{-1} (KBr and solution) and in 2-pyrrolidone at 1690 cm^{-1} (KBr). In solution the unknown exhibited an absorption at 1605 cm^{-1}. This band was not present in the solid state i.r. spectrum. Its frequency is too high for amide II absorption and is more consistent with a C=N deformation. Also, cyclic lactams with less than nine membered rings do not exhibit amide II absorption.[53] The fact that this absorption occurred only in solution suggested that (49) or (50) could be in equilibrium with the open chain form (51). This compound could also arise from dehydrobromination of (48), and ought to be particularly susceptible to hydrolysis. However, acid hydrolysis of the unknown in aqueous dioxan only removed the ethylene ketal group and the absorption at 1605 cm^{-1} was still present in the i.r. spectrum of the product. Alkaline hydrolysis gave a mixture of many products, none of which were identified.

The u.v. spectrum of the unknown showed an absorption maximum at 213 nm (ϵ, 3,800). Addition of sodium hydroxide resulted in two maxima (218 nm and a broad shoulder 230 nm), both of increased intensity. Reprotonation gave a different species from the original. In contrast, the u.v. spectrum of


(48+)
-HBr
(49+)
-HNO2
(51+)
-HNO2
M'
(53)
m/e 381
C19H27NO7
(54)
m/e 381
C19H27NO7
m/e 295
m/e 282
M' - C(CO2Et)2
m/e 223
m/e 99
m/e 86

SCHEME VIII

acetamidodiethylmalonate (52) showed a weak transition at around 210 nm (ϵ, 760) but on addition of base, two strong absorptions at 215 nm and 270 nm appeared. This evidence suggested that although the unknown contained acidic protons, the structure (50) could prcbably be omitted on the basis of the u.v. data.

The mass spectrum of the unknown confirmed that it consisted of at least two components. In the spectrum of the most volatile component, a large proportion of the ion current was carried by fragments m/e 86 and m/e 99, both characteristic of cyclic ethylene ketals. There was no indication of bromine in this fraction and a molecular ion could not be observed. Ion m/e 381 (accurate mass $\equiv C_{19}H_{27}O_7$) is significant, although it may have arisen from either (48) or (49) as shown in Scheme VIII. From $(49)^+$ loss of HNO_2 leads to m/e 381. Two steps are required from $(48)^+$ - first loss of HBr, then loss of HNO_2. The remaining fragmentation pattern does not enable an unambiguous assignment as to whether the parent ion was (53) or (54).

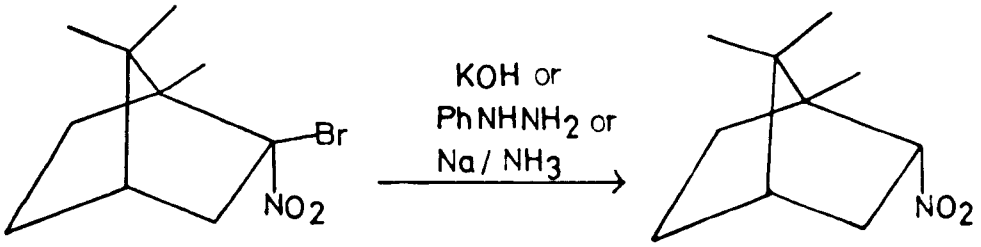
The least volatile fraction contained bromine, as indicated by ions m/e 479 and m/e 481. The corresponding ion less bromine (m/e 400) was very weak . The base peak was again m/e 99 and the other major current carrying ion was m/e 86, both indicating an ethylene ketal. The remaining fragmentation pattern was not consistent with any of the expected cyclised products and was considerably different from that of the starting material (30).

In conclusion, evidence suggested that a clean, successful coupling reaction was unlikely, since halogen exchange appeared to be a serious side reaction. Also from the evidence available we could not unequivocally ascertain whether cyclisation of this model compound (30) had occurred or not. The usefulness of this approach did not appear great enough to warrant a synthesis of a similar model containing the ring D portion.

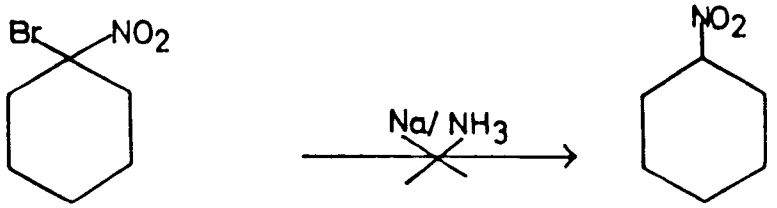
Some Related Reactions of Bromonitro Compounds

Dehalogenation Reactions

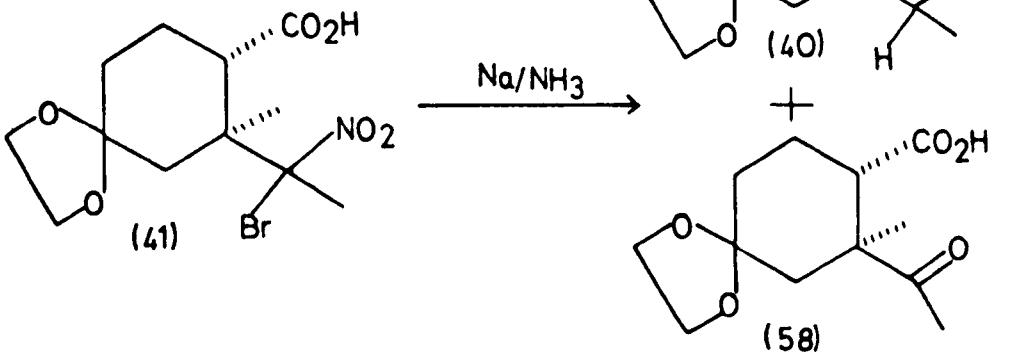
The fact that gem bromonitro compounds can be dehalogenated by certain reagents was a serious drawback to our attempts to use them in coupling reactions with anions. This observation has been put to some use by Forster,[54] who converted 2,2-bromonitrobornane (55) to 2-nitrobornane (56) by reaction with potassium hydroxide. However, this was not a general method for the preparation of nitro

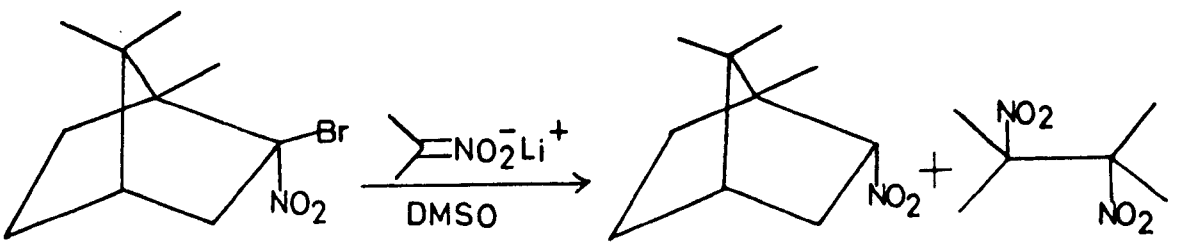

KOH or
PhNHNH2 or
Na/ NH3
Br
NO2
NO2

(55) (56)


Br
NO2
Na/ NH3
NO2

(57)


CO2H
O
O
NO2
Br
Na/NH3
CO2H
O
O
NO2
H
(40)
+
CO2H
O
O
O
(41)
(58)


Br
NO2
=NO2-Li+
DMSO
NO2
+
NO2
NO2

compounds. Forster also found that phenylhydrazine converted 2,2,-bromonitrobornane to the nitro compound in good yield.[55]

From interest in the syntheses of aliphatic nitro compounds, methods were examined for their preparation from bromonitro compounds. The Iffland method,[56] which uses a large excess of sodium borohydride for debromination, is not always reliable. An improvement on this reaction would be desirable, since bromonitro compounds are fairly readily available from the corresponding oximes. Phenylhydrazine reacted with 2,2,-bromonitro-3,3-dimethylbutane (20), giving about 20% 2-nitro-3,3-dimethylbutane with recovered starting material. Conditions were not discovered whereby this reaction proceeded to completion.

Dissolved metals in liquid ammonia are powerful reducing agents. 2,2-Bromonitrobornane in liquid ammonia was converted in good yield to 2-nitrobornane by two equivalents of sodium. A similar reaction with 1,1-bromonitrocyclohexane (57) resulted in a complex mixture of products and no nitrocyclohexane was produced. In this case it is probable that the initially formed radical-anion reacted with the bromonitro compound to give coupled products. Where there are steric constraints about the bromonitro centre, coupling fails to occur and dehalogenation proceeds to completion. The nitronate anion formed is then protonated to give the nitro compound on work up. This argument was confirmed by reacting the highly hindered ketal bromonitro acid (41) with sodium in liquid ammonia: the ketal nitro acid (40) was the major product. Some of the ketone (58) was also produced and it is possible that this arose <u>via</u> rearrangement of a nitro radical as discussed earlier in this chapter. The results of these latter experiments were relevant to the failures encountered in attempts to couple nitronate anions with hindered bromonitro compounds.

Secondary nitronate anions react with simple gem halogenonitro compounds. Our observations suggested that a secondary nitronate anion would not react with 2,2-bromonitrobornane (55) to give coupled products due to steric interactions. Reassuringly (or unfortunately!) this turned out to be the case. Reaction of 2,2-bromonitrobornane with lithium 2-propane nitronate in DMSO formed mainly 2-nitrobornane (some 2-bornanone oxime was also isolated) and 2,3-dimethyl-2,3-dinitrobutane. This latter product probably arose from bromine transfer yielding 2,2-bromonitropropane which then coupled with unreacted 2-propanenitronate anion.

These results compounded the observations concerning the failure of primary nitronate anions to react cleanly with bromonitro compounds to give useful coupled products. Compelling evidence was now available indicating that a coupling reaction to join ring A and ring D intermediates, whether intermolecular or intramolecular, was unlikely to be in these approaches.

EXPERIMENTAL I

Preparation of Primary Nitronate Salts

Lithium 1-propane nitronate

The general procedure of Kornblum[57] was used.

Lithium hydride (0.78 g, 98 mM) was added to anhydrous absolute EtOH (100 ml), and stirred for 15 minutes under a dry N_2 atmosphere until a clear solution resulted. 1-Nitropropane (9.0 g, 101 mM) was added dropwise and stirred for 10 minutes. Et_2O (200 ml) was added causing precipitation of the white nitronate salt which was filtered under N_2 and washed with anhydrous ether. The salt was vacuum dried for 48 hours, then stored under N_2 (5.5 g, 57%).

Lithium 2-propane nitronate

Lithium 2-propane nitronate was prepared as described by Kornblum.[57]

Thallium 1-propane nitronate (TPN)

Thallium ethoxide was stored at - 20° C. Prior to its use it was allowed to melt at room temperature, then rapidly filtered through Celite under an N_2 stream. Thallium ethoxide (6.53 g, 26.2 mM) was quickly added dropwise in a stream of N_2 to a stirred solution of 1-nitropropane (2.35 g, 26.4 mM) in dry freshly distilled THF (50 ml). The suspension was stirred for a further 10 minutes, filtered, and the solid washed with dry THF. The salt was dried *in vacuo* to yield bronze-brown platelets (7.0 g, 91%) which were stored at - 20° C. (Note : the colour of the crystals varied from dark brown to light bronze in several preparations, although the infrared spectra of all the preparations were identical).

Preparation of Bromonitro Compounds

2,2-Bromonitropropane and 2,2-bromonitro-3,3-dimethylbutane and 2,2-bromonitrobornane were prepared from the oximes by the Iffland procedure.[56] The following description is typical.

2,2-Bromonitro-3,3-dimethylbutane (20)

A suspension of pinacolone oxime (4.0 g, 34.8 mM) and $NaHCO_3$ in water (35 ml) was added portionwise to a well stirred mixture of N-bromosuccinimide in water (40 ml)/dioxan (40 ml) cooled in ice. Stirring was continued for 2 hours after addition was completed. The mixture was extracted with pentane, then the solvents were removed *in vacuo* until the volume of the solution was about 25 ml.

The blue solution was cooled in ice, and 70% HNO_3 (15 ml), followed by 100 vol H_2O_2 (7 ml) were added. The mixture was vigorously stirred at room temperature until the blue colour disappeared. The organic layer was separated, washed with aqueous $NaHCO_3$, water, then dried ($MgSO_4$). Removal of solvent gave a white lachrymatory solid which was recrystallised from absolute EtOH.

m.p.	195 - 197° C
n.m.r. (CCl_4) :	δ = 1.22 (S, 9H), 2.5 (S, 3H)
i.r. (CH_2Cl_2) :	ν cm^{-1} 2960 m 2925 sh, 2895 m, 1550 vs

Preparation of Halogenonitroso Compounds

2,2-Bromonitroso-3,3-dimethylbutane[38]

Pinacolone oxime (3.45 g, 30mM) was dissolved in pyridine (30 ml) and water (75 ml) and cooled to 0° C under N_2, giving an emulsion. Bromine (5.8 g, 30mM) was added dropwise during 5 minutes with vigorous stirring to disperse the brown pyridinium salt that formed. Reactants were protected from the light to avoid any photochemical decomposition of the product. The suspension was stirred for 40 minutes at 0° C then the blue liquor was quickly decanted to avoid oxidation of the bromonitroso compound that had formed. The mixture was then extracted with pentane and extracts were washed with 2NHCl, water, until washings were neutral, then dried ($MgSO_4$). The solvent was removed in vacuo with some care as the product was particularly volatile (even so, some was lost in the rotary evaporator). The intensely blue residue was purified by silica gel filtration (eluant 10% CH_2Cl_2/pentane), yielding a powerfully lachrymatory blue crystalline solid (3.3 g, 57%) which was pure by n.m.r. and one component by t.l.c. The product was stored under N_2 at - 20° C and protected from the light.

n.m.r. ($CDCl_3$):	δ = 1.30 (S, 9H), 1.65 (S, 3H)
i.r. (CH_2Cl_2) :	ν cm^{-1} 2970 m, 2910 m, 2880 m, 1578 s.

2,2-Chloronitroso-3,3-dimethylbutane

t-Butylhypochlorite[58] (1.19 g, 1.24 ml, 11 mM) was added dropwise to a solution of pinacolone oxime in CF_3Cl (25 ml) under N_2 and protected from the light. The reaction was exothermic and cooling was necessary to prevent the

solvent boiling. The blue solution was stirred for 35 minutes at room temperature and then solvent removed in vacuo to give a blue solid (1.3 g). The crude material was dissolved in the minimum amount of pentane and subjected to silica gel filtration, eluting with pentane. Only the first blue fraction was collected (770 mg 52%).

m.p.	:	120 - 122° C (lit. 122°)[41]
n.m.r. ($CDCl_3$):		δ 1.28 (S, 9H), 1.64 (S, 3H)
i.r. (CH_2Cl_2)	:	ν cm^{-1} 2940 m, 2910 m, 2875 m, 1580 vs

Reactions of TPN with 2,2-Bromonitropropane

A typical procedure was as follows : TPN (510 mg, 1.74 mM) was flushed with dry N_2 for 30 minutes then suspended in dry MeCN (10 ml). 2,2-bromonitropropane (168 mg, 1 mM) was added via a rubber septum and the stirred mixture irradiated with a 150 W tungsten lamp maintaining the temperature at 50° C. Reaction was evident when the brown thallium salt became orange. The course of the reaction was followed by settling the reactants and removing aliquots periodically. Samples were filtered through Celite and the MeCN removed in vacuo. Et_2O was added to precipitate thallium compounds which were filtered onto Celite. Solvent was removed in vacuo and the above procedure was repeated until no thallium residues were present in the product. The proportions of products and starting material in the isolated mixture were estimated by ^1H n.m.r.

Experiments to examine the effects of temperature, irradiation, reaction time and solvent were performed using a similar technique to that described above, varying one parameter at a time.

Attempted Preparation of 2-Methyl-3-nitro-pent-2-ene (13) from 2,2-Bromonitropropane

2,2-bromonitropropane (1.36 g, 8.1 mM) was reacted with TPN (4.1 g 14.0 mM) in MeCN (100 ml) at 50° C under N_2 using conditions outlined above. Stirring and irradiation were continued for 24 hours, although it was noted that little change in the product mixture occurred after 11 hours. The reaction mixture was filtered, then worked up using the described procedure to yield a brown oil (867 mg). T.l.c. (Sigel; 1:1 CH_2Cl_2/petrol (40 - 60°) indicated at least four components. The oil was crystallised from pentane (-78° C) to yield 2,3-dinitro-

2,3-dimethylbutane (110 mg, 8%) m.p. 212 - 214° (lit. 215.5 - 216)[18] (n.m.r. ($CDCl_3$) δ = 1.70 (S)). The residue was distilled in vacuo (80° / 0.01 mm) but separation was not effected. The crude distillate was subjected to p.l.c. (Sigel; 1 : 1 CH_2Cl_2 / petrol (40 - 60°)) but good separation was not entirely achieved. A semicrystalline material (50 mg) (not analytically pure), whose spectroscopic properties were in accordance with 2-methyl-3-nitro-pent-2-ene was obtained.

n.m.r. (CCl_4) : δ = 1.10 (t, 7, 3H), 1.88 (S, 3H), 2.50 (m, 2H)
i.r. (film) : νcm^{-1} 2980 m, 2940 m, 2880 m, 1550 s, 1515 s

Reaction of TPN with 2,2-Bromonitro-3,3-dimethylbutane

To a stirred suspension of TPN (510 mg, 1.74 mM) in dry MeCN (10 ml) under N_2, 2,2-bromonitro-3,3-dimethylbutane in dry MeCN (5 ml) was added. The suspension was stirred at 55° C with irradiation from a 150W tungsten lamp for 5 hours.

The mixture was filtered and the filtrate divided into 2 equal aliquots. The first aliquot was evaporated and worked up as usual to yield a crystalline solid whose spectroscopic data and mixed melting point confirmed its identity as unreacted bromonitro compound. The equivalent of > 85% unreacted starting material was recovered. (Further reaction of starting material did not occur in a separate experiment maintained at 70° C). 2,4-Dinitrophenylhydrazine solution[59] was added to the second aliquot until no more precipitate formed. The precipitate was extracted with benzene and concentrated. Comparison with authentic pinacolone 2,4-DNP using t.l.c. (Neutral Alumina; 3 : 5 Et_2O / petrol (40 - 60°) or Sigel; 3 : 5 Et_2O / petrol) confirmed the formation of pinacolone in the reaction mixture. Absorptions in the i.r. spectrum of the crude reaction mixture corresponded to those of pinacolone (C = O, νcm^{-1} 1705 s).

Reaction of Lithium 1-Propanenitronate with 2,2-Bromonitro-3,3-dimethylbutane

Lithium 1-propanenitronate (166 mg, 1.73 mM) was placed in the reaction vessel and the system swept with dry argon for ½ hour before the salt was dissolved in dry DMSO (6 ml). 2,2-Bromonitro-3,3-dimethylbutane (210 mg, 1.0 mM) in dry DMSO (6 ml) was added via a rubber septum to the nitronate salt solution, maintained under static argon pressure. The solution was stirred at room temperature

irradiating with a 150W tungsten lamp, and became deep red within ca. 5 minutes. After 1 hour t.l.c. (Sigel; CH_2Cl_2) showed no starting material. Ice-water was added followed by extraction with Et_2O. Extracts were washed well with water and dried ($MgSO_4$). Evaporation yielded a yellow oil (85 mg), the n.m.r. spectrum of which was complex, but signals corresponding to those of 2-nitro-3,3-dimethylbutane could be identified (n.m.r. (CCl_4) δ = 1.04 (S, 9H), 1.58 (d, 7, 3H), 4.35 (q, 7, 1H).

The aqueous solution was acidified to pH 3 with dilute HCl, followed by extraction with Et_2O. Washing, drying and evaporation of extracts gave a yellow oil (65 mg) which was a mixture of compounds. The n.m.r. spectrum showed the characteristic low field triplet (δ = 6.16, J = 7) of 1,1-bromonitropropane.

Reaction of Lithium 1-Propanenitronate with 2,2-Bromonitroso-3,3-dimethylbutane

Lithium 1-propanenitronate (138 mg, 1.45mM) was dissolved in dry DMSO (5 ml) after the usual $\frac{1}{2}$ hour flushing with argon. 2,2-Bromonitroso-3,3-dimethylbutane (163 mg, 0.84mM) in DMSO (5 ml) was added and the solution was irradiated at room temperature. The blue colour of the nitroso compound immediately disappeared and there was a slight exotherm to 35° C. An intense red solution resulted and there were no starting materials present after 10 minutes. After 20 minutes, ice-water was added and the solution was extracted with pentane. Washing with water, drying ($MgSO_4$) and evaporation gave a colourless crystalline solid (40 mg). Its n.m.r. (($CDCl_3$) : δ = 1.03 (S, 9H), 1.88 (S, 3H)) and i.r. spectra confirmed its identity as pinacolone oxime, on comparison with those of authentic material.

Acidification of the aqueous solution with dilute HCl, followed by further extraction, gave a yellow oil (100 mg) which contained mainly pinacolone oxime and some 1,1-bromonitropropane (n.m.r. ($CDCl_3$) : δ = 6.16,t,J = 7).

Reaction of Lithium 1-Propanenitronate with 2,2-Chloronitroso-3,3-dimethylbutane

2,2-Chloronitroso-3,3-dimethylbutane (149.5 mg, 1 mM) in DMSO (6 ml) was added to lithium 1-propanenitronate (165 mg, 1.73mM) (previously flushed with argon in the usual way) in DMSO (6 ml), and irradiated at room temperature. The initial blue solution quickly became more intense in colour, although no starting materials were present after 15 minutes. If the reaction was terminated after this time by addition of ice-water and worked up in the usual way, the only identifiable

product was pinacolone oxime. Allowing reaction to proceed until the blue colour had faded completely (2 hours), followed by the usual work up, resulted in a mixture of products, none of which could be identified.

A reaction of 2,2-chloronitroso-3,3-dimethylbutane with one equivalent of lithium 1-propanenitronate in $^{2}H_{6}$-DMSO followed by ^{1}H n.m.r. indicated that pinacolone was also a product. N.m.r. $(CD_3)_2SO$ δ = 1.09 (S, 9H), 2.09 (S, 3H).

Reaction of 2,2-Bromonitrobornane with Sodium-Liquid Ammonia

2,2-Bromonitrobornane (1.31 g, 5mM) in dry THF (10 ml) was added to dry, freshly distilled liquid ammonia (50 ml) at -33°C. Sodium (230 mg, 10mM, clean freshly cut) was added in small pieces during 20 minutes. After each addition the characteristic blue colour appeared, and when reaction was complete the solution was colourless. Stirring was continued for a further 10 minutes by which time the solution was viscous and some precipitation had occurred. Ammonia and solvent were removed in a stream of N_2. The resulting white solid was dissolved in water and aqueous KOH was added to raise the pH to ~13. The solution was extracted with Et_2O (53 mg, unidentifiable yellow oil) and then acidified to pH 4 with 15% hydroxylamine hydrochloride. Extraction with Et_2O, drying and evaporation, gave a colourless crystalline solid (738 mg) which was at least 85% 2-nitrobornane (n.m.r.) (69% yield). Comparison with spectral data for authentic material confirmed its identity. Some 2-bornanone was also identified. N.m.r. (CD_3OD) 100 MHz : δ = 0.96 (S, 6H), 1.08 (S, 3H), 0.90 - 1.86 (m, 5H), 2.20 (m, 2H), 4.84 (m, $W_{\frac{1}{2}}$ = 17, 1H).

Reaction of 2,2-Bromonitrobornane with Lithium 2-propanenitronate

Lithium 2-propanenitronate (332 mg, 3.46mM) was flushed with argon and dissolved in dry DMSO (10 ml). 2,2-Bromonitrobornane (524 mg, 2mM) in DMSO (10 ml) was added in the usual way. The reaction was irradiated and stirred at room temperature; after 2 hours no starting material was present. Ice-water was added and the solution extracted with Et_2O. Washing with water, drying and evaporation gave a crystalline solid (353 mg) which was a mixture of mainly 2-nitrobornane, 2,3-dimethyl-2,3-dinitrobutane and a little bornanone oxime. The crude material was recrystallised from EtOH yielding pure 2,3-dimethyl-2,3-

dinitrobutane (150 mg, 43%). (N.m.r. ($CDCl_3$) 100 MHz : δ = 1.75 (S)). 2-nitrobornane and bornanone oxime were separated from the residue by chromatography on Sigel (CH_2Cl_2) and were identified by comparison of their spectral data with that of authentic materials.

The aqueous solution was acidified to pH 4 with 15% aqueous hydroxylamine hydrochloride, and extracted as before to give a green oil (102 mg). T.l.c. (Sigel; 1 : 4 CH_2Cl_2/pentane) indicated a complex mixture of products from which all of those previously noted could be identified.

Reaction of 1,1-Bromonitrocyclohexane with Sodium-Liquid Ammonia

1,1-Bromonitrocyclohexane (1.04 g, 5mM) in dry THF (5 ml) was dissolved in dry distilled liquid ammonia (100 ml). Sodium (230 mg, 10mM) was added in small pieces during 20 minutes with stirring. After a further 10 minutes the ammonia and solvent were removed in a stream of N_2. Water was added to the residue and the solution basified to about pH 13 with aqueous KOH. Extraction with Et_2O gave a yellow oil (219 mg) which was a mixture of products and not identified. Acidification of the aqueous solution with 15% aqueous hydroxylamine hydrochloride followed by extraction, gave a brown oil (137 mg) which was not identified, but did not contain any nitrocyclohexane.

Reaction of bromonitrocyclohexane with 10 equivalents of sodium in liquid ammonia followed by quenching of the excess metal with EtOH, resulted in an identical complex mixture of products. No nitrocyclohexane could be identified.

Reaction of Ketal Bromonitro Acid (41) with Sodium-Liquid Ammonia

Ketal bromonitro acid (41) (352 mg, 1 mM) in dry THF (2 ml) was added to dry distilled liquid ammonia (10 ml) followed by sodium (93 mg, 4mM) in small portions. Stirring was continued 10 minutes after the addition, and the blue colour removed by addition of EtOH. Water was added (pH 13) and the solution extracted with ether (10 mg,oil). 15% aqueous hydroxylamine hydrochloride was added to the aqueous solution, which was ether extracted to yield a crystalline solid (153 mg). N.m.r. indicated that this material contained ~90% ketal nitro acid (24) (50% yield) and some of the ketone acid (58). Products were identified by comparison of their spectral data with those of authentic materials.

Synthesis of 4-Carboxamidodiethylmalonyl-3-methyl 3-(2',2'-bromonitroethyl)cyclohexanone-ethylene Ketal

Ethyl Hagemann's Ester

Base catalysed addition of ethyl acetoacetate to methyl vinyl ketone gave the dioxo ester (16), which was cyclised to ethyl Hagemann's ester by refluxing with a catalytic quantity of pyrrolidinium acetate.

Michael Addition of Nitroethane to Ethyl Hagemann's Ester[17]

Hagemann's ester (137 g, 0.753 M), nitroethane (140 g, 1.87 M) and benzyltrimethyl ammonium ethoxide[60] (165 ml of a freshly prepared 1.5 M solution in ethanol) were stirred at 40° C under N_2 for 110 hours. The formation of products was followed by ^1H n.m.r. At equilibrium, Et_2O (500 ml) was added and the solution acidified to pH 3 with 1 N HCl. The mixture was extracted with CH_2Cl_2, and the combined extracts washed with dilute sodium hydroxide, then brine, and dried ($MgSO_4$). Removal of solvent yielded a red-brown viscous oil which was fractionally distilled under reduced pressure to give nitroethane, Hagemann's ester, and the nitroester adducts (34a, 34b) (70.5 g 38%, b.p. 154 - 158° C / 0.2 mm). Addition of Et_2O / pentane and cooling (-78° C) induced crystallisation of one epimer (13 g), which was recrystallised (2x) from ether / pentane.

m.p.	:	91 - 92°
n.m.r. ($CDCl_3$)	:	δ = 1.15 (S, 3H), 1.31 (t, 7, 3H), 1.48 (d, 7, 3H), 2.05 - 2.90 (m, 7H), 4.17 (q, 7, 2H), 4.96 (q, 7, 1H)
i.r. (CH_2Cl_2)	:	ν cm^{-1} 2900 m, 1710 - 1720 s, 1544 s, 1353 m, 1194 s, 1114 m, 1034 s, 865 w
u.v. (MeOH)	:	282 nm

The mother liquors contained almost entirely the liquid epimer nitroester, which was a viscous oil.

n.m.r. ($CDCl_3$)	:	δ = 1.09 (S, 3H), 1.31 (t, 7, 3H), 1.56 (d, 7, 3H), 2.10 - 2.90 (m, 7H), 4.19 (q, 7, 2H), 4.67 (q, 7, 1H)

Equilibration of the Liquid Epimer

Liquid nitroethyl ester (34a, 15g) was refluxed with triethylamine (15 ml) in carbon tetrachloride (50 ml) for 10 minutes, then cooled and stored at - 20° C. After one week the crystals were filtered off, and dissolved in dry ether and filtered again to remove some triethylamine hydrochloride. Removal of solvent

gave the crystalline nitroester (34b, 5 g). Further storage of the residue caused deposition of more crystals.

Preparation of Ethylene Ketal Nitroester (39)

Crystalline nitroethyl ester (34b) (10 g, 39 mM), ethanediol (3.5 g, 57 mM) and a catalytic amount of p-toluene sulphonic acid (270 mg) were refluxed in benzene (250 ml) for 24 hours using a Dean and Stark distillation head for water removal The solution was cooled, washed with dilute K_2CO_3, water, then brine and dried ($MgSO_4$). Evaporation yielded a white crystalline solid (11.6 g, 99%) which was a single component by t.l.c. Recrystallisation from EtOH gave analytically pure material.

m.p. : 75 - 76° C

n.m.r. ($CDCl_3$) : δ = 1.20 (S, 3H superimposed on t, 7, 3H), 1.46 (d, 7, 3H), 1.60 - 2.05 (m, 6H) 2.10 - 2.48 (m, 1H), 3.90 (S, 4H), 4.10 (q, 7, 2H), 4.87 (q, 7, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 2950 m, 2900 m, 1725 m, 1544 s, 1353 s, 1173 s, 1100 s, 1030 m, 950 m

		% C	H	N
$C_{14}H_{23}NO_6$	requires	55.80	7.69	4.65
	found	56.00	7.43	4.73

Using the nitroethyl ester which was predominantly the liquid epimer (34a), a liquid ethylene ketal nitroester was obtained. Its n.m.r. was identical to the crystalline epimer, although crystallisation could not be induced. The ketal was purified by column chromatography (2.0 g product / 100 g Silica gel. eluting with CH_2Cl_2, followed by 0.5 - 2.0% MeOH / CH_2Cl_2).

Preparation of the Ketal Nitro Acid (40)
by Alkaline Hydrolysis of (39)

Crystalline ethylene ketal nitroester (39) (450 mg, 1.5 mM) was refluxed with aqueous 2N NaOH (15 ml) in MeOH (15 ml) for 4 hours. The solution was cooled and dilute acetic acid added to pH 9. Neutral material (3 mg) was removed by extraction with Et_2O. The aqueous solution was acidified with dilute acetic acid to pH 4 and then extracted with CH_2Cl_2. The extracts were combined and washed well with water, brine, and dried ($MgSO_4$). Removal of solvent gave a white crystalline solid (385 mg, 94%) which was a single component by t.l.c., and a single epimer by n.m.r. Recrystallisation from Et_2O / pentane gave colourless

crystals.

m.p. : 156 - 157° C

n.m.r. ($CDCl_3$) : δ = 1.25 (S, 3H), 1.35 (d, 7, 3H), 1.60 - 2.20 (m, 6H), 2.30 - 2.55 (m, 1H), 3.90 (S, 4H), 5.15 (q, 7, 1H), 9.70 (variable δ brS, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3470 m, 2960 m, 2885 m, 1735 s, 1705 s, 1545 s, 1095 s

	% C	H	N
$C_{12}H_{19}NO_6$ requires	52.74	7.01	5.13
found	53.00	6.84	5.22

Preparation of Ethylene Ketal Nitronic Acid (43)

To a solution of the crystalline ethylene ketal nitroester (39b) (100 mg, 0.33 mM) in MeOH (1 ml) was added sodium hydroxide (41.5 mg, 1.04 mM) in water (0.5 ml). The mixture was briefly warmed to 50° C to effect complete solution, then stirred at ambient temperature for 5 minutes. Dilute acetic acid was added, adjusting the pH to ~ 9 followed by Et_2O extraction to remove any neutral material (zero). The aqueous solution was acidified to pH 4 with dilute acetic acid and the resulting white precipitate filtered, washed with water, ether, then dried in vacuo. The white solid nitronic acid (88 mg, 88%) showed considerable decomposition after storage at room temperature for one day. It also rapidly decomposed in hot ethanol. It was more stable when stored at sub-zero temperatures.

m.p. : 75 - 76° C (decomp.)

n.m.r. ($CDCl_3$) : δ = 1.20 (t, 7, 3H), 1.30 (S, 3H), 2.08 (S, 3H), 1.45 - 2.20 (m, 7H), 4.90 (S, 4H), 4.05 (q, 7, 2H), 5 - 10 (brS, variable chemical shift, 1H)

Low field acidic H exchanges in CD_3OD

i.r. (CH_2Cl_2) : νcm^{-1} 3460 br m, 2940 m, 2890 m, 1725 s, 1645 m, 1475 m

u.v. (MeOH) : 229 nm (ϵ 7,800)

Bromination of Ethylene Ketal Nitro Acid (40)

Ethylene ketal nitro acid (40) (5.45 g, 19.95 mM) and freshly sublimed potassium t-butoxide (4.95 g, 44.2 mM) were stirred 15 minutes in dry t-butanol (136 ml) until the solution was homogeneous. Bromine, freshly distilled from CsBr (3.74 g, 23.4 mM) was added dropwise until the white suspension became faint orange in colour. Stirring was continued for a further 10 minutes and then

the solvent evaporated in vacuo. Water was added and the solution acidified with dilute HCl to pH 3, then extracted with EtOAc. Combined extracts were washed with water, brine, then dried ($MgSO_4$). Removal of solvent yielded a pale yellow solid (6.8 g) which was recrystallised from CH_2Cl_2/pentane to give pure bromonitro acid (41) (4.0 g, 57%). The residue contained a considerable amount of bromonitro acid but this failed to crystallise.

m.p.	:	170 - 171° C
n.m.r. ($CDCl_3$)	:	δ = 1.50 - 1.95 (m, 6H), 1.64 (S, 3H), 2.35 (S, 3H), 2.60 - 2.90 (m, 1H), 4.90 (S, 4H), 8.00 (S, 1H)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3480 m, 2950 m, 2880 m, 1745 s, 1710 s, 1553 s, 1380 m
i.r. (KBr)	:	νcm^{-1} 3400 - 3000 br m, 2920 m, 2890 m, 1710 s, 1710 s, 1695 sh s, 1553 s, 1390 s
M.S.	:	235 (72), 202 (40), 199 (40), 158 (36), 100 (27), 99 (B), 86 (100), 85 (72), 83 (100), 67 (18), 55 (27), 44 (39), 43 (72), 41 (24)

		% C	H	N	Br
$C_{12}H_{18}BrNO_6$	requires	40.93	5.15	3.97	22.70
	found	41.02	5.17	3.88	22.54

Preparation of Bromonitro Acid Chloride (42)

Freshly distilled thionylchloride (10.1 g, 85 mM) was added to a suspension of the ketal bromonitro acid (41) (1.5 g, 4.26 mM) in dry benzene (31 ml) under N_2. Stirring was continued for 26 hours at 37° C, with protection against moisture, until a homogeneous solution resulted. Periodic examination by i.r. spectroscopy indicated when reaction was complete. Benzene and excess thionylchloride were removed in vacuo, affording a yellow oil (1.58 g, 100%) which was pure by n.m.r. and the i.r. spectrum showed no unreacted acid.

n.m.r. ($CDCl_3$)	:	δ = 1.54 (S, 3H), 2.05 - 2.64 (m, 6H), 3.20 - 3.50 (m, 1H), 4.90 (S, 4H)
i.r. (film)	:	νcm^{-1} 2960 m, 2900 m, 1800 s, 1553 s, 1385 m
M.S.	:	339 (2), 290 (4), 225 (32), 217 (37), 181 (27), 139 (12), 101 (47), 99 (B), 86 (96), 67 (37), 55 (75), 43 (75), 43 (76), 42 (43), 41 (57)

Preparation of Ethylene Ketal Bromonitroamide (30)

Diethylaminomalonate hydrochloride[61] (900 mg, 4.26 mM) and N-methyl

morpholine (430 mg, 4.26 mM) in dry CH_2Cl_2 (17 ml, distilled from P_2O_5) were added to a stirred solution of the bromonitro acid chloride (42) (1.58 g, 4.26 mM) in dry CH_2Cl_2 (12.5 ml) under N_2. After 5 minutes a second equivalent of N-methyl morpholine (430 mg, 4.26 mM) in dry CH_2Cl_2 (17 ml) was added. The reaction mixture was stirred for $1\frac{1}{4}$ hours following the disappearance of the acid chloride by i.r. spectroscopy. The solution was washed with 1N HCl, water, then brine, and dried ($MgSO_4$). Evaporation gave a viscous yellow oil (1.97 g) which crystallised from Et_2O/pentane to yield the bromonitroamide (30) as white crystals (1.4 g, 65%). Filtration through silica gel (for p.l.c.) eluting first with benzene, CH_2Cl_2, then 2% MeOH / CH_2Cl_2, and recrystallisation from Et_2O/pentane, gave analytically pure material.

m.p.	:	98 - 99°C
n.m.r. ($CDCl_3$)	:	δ = 1.30 (t, 7, 6H), 1.62 (br S, 6H), 1.82 (br S, 3H), 2.35 (S + sh, 4H), 4.25 (q, 7, 4H), 5.05 (d, 7, 1H), 6.6 (br d, 7, 1H)
i.r. ($CHCl_3$)	:	νcm^{-1} 3420 m, 2950 m, 2890 m, 1760 - 1740 s, 1680 s, 1550 s, 1495 s
i.r. (KBr)	:	νcm^{-1} 3300 m, 2980 m, 2955 m, 2890 m, 1758 s, 1730 s, 1660 s, 1552 s, 1535 s, 1443 m
M.S.	:	479 (18), 398 (22), 357 (22), 356 (90), 315 (18), 313 (24), 225 (41), 181 (28), 179 (55), 178 (41), 176 (43), 154 (22), 139 (55), 137 (47), 102 (55), 99 (97), 86 (B), 67 (52), 55 (97), 43 (78), 41 (59)

		% C	H	N	Br
$C_{19}H_{29}BrN_2O_9$	requires	44.78	5.74	5.50	15.69
	found	45.07	5.88	5.48	15.44

Reactions of Bromonitroamide (30) under Basic Conditions

(i) With Sodium Ethoxide in Ethanol

Sodium ethoxide was prepared from anhydrous ethanol and sodium, cleaned by brief immersion in ethanol. The solution was standardised with hydrochloric acid prior to use.

Ketal bromonitroamide (30) (203.6 mg, 0.4 mM) was flushed with argon for 30 minutes and then dissolved in anhydrous EtOH (1.6 ml). Sodium ethoxide (190 µl of a 2.32 M solution in EtOH, 0.44 mM) was added via a rubber septum and the solution stirred under an argon atmosphere for 2 hours at room temperature.

Solvent was then removed in vacuo and water added to the residue. The aqueous suspension was neutralised with dilute HCl and filtered, washing well with water and then dried in high vacuum (0.1 mm). The product was a white amorphous solid (184 mg) which was recrystallised with difficulty from Et_2O/pentane (-78° C). The material failed to crystallise from other solvents. (Separate experiments were carried out to follow the course of the reaction with time, using infrared spectroscopy and t.l.c. (Sigel; EtOAc; Et_2O/benzene; $CHCl_2$; 1% MeOH/CH_2Cl_2). For infrared measurements, aliquots were removed periodically and evaporated. The residue was diluted with water, neutralised with 1N HCl, and extracted (CH_2Cl_2). Measurements were taken on the dried and concentrated solution).

m.p.	:	Softened 65° C, Melted 80 - 95° C
n.m.r. ($CDCl_3$)	:	δ = 1.25 (t, 7, 6H), 1.50 - 2.40 (m, 14H), 3.90 (S, 4H), 4.25 (q, 7, 4H), 5.05 (m, $\frac{1}{2}$H), 7.25 (br S)
$(CD_3)_2CO$	:	δ = 1.25 (t, 7, 6H), 1.50 - 2.40 (m, obscured by residual $(CH_3)_2CO$), 4.90 (S, 4H), 4.20 (q, 7, 4H), 5.15 (m, $\frac{1}{2}$H), 7.90 (br S, $\frac{1}{2}$H)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3400 br m, 2950 m, 2900 m, 1750 s, 1690 s, 1605 m, 1550 s, 1370 w, 1225 m, 1195 w, 1105 m, 1025 m, 955 w, 865 w
(KBr)	:	νcm^{-1} 3380 br m, 2960 m, 2880 m, 1750 s, 1692 s, 1550 s, 1500 m, 1390 m, 1368 m, 1300 m, 1253 m, 1185 w, 1100 m, 1022 m, 950 m, 858 m
u.v. (MeOH)	:	213 nm (ϵ, 3,800)
(MeOH/HCl)	:	213 nm
(MeOH/NaOH)	:	218 , br sh 230 nm
M.S.	:	Two fractions
(i)		m/e 401 (0.5), 381 (6.1), 366 (0.5), 336 (1.0), 308 (1.4), 295 (4.2), 282 (2.8), 281 (2.8), 256 (2.5), 223 (14), 181 (4), 154 (32), 129 (4), 124 (17), 99 (B), 86 (100), 74 (30), 58 (75), 55 (30), 45 (73), 43 (100)
Acc. Mass.	:	m/e 381.1789 ($C_{19}H_{27}NO_7$ = 381.1788)
(ii)		481 (1.6), 479 (1.8), 444 (1.4), 428 (0.9), 401 (15), 381 (4), 354 (3.5), 324 (2.5), 256 (25), 225 (26), 209 (7), 181 (20), 154 (16), 146 (33), 129 (17), 107 (15), 99 (B), 86 (67), 80 (7), 55 (22), 43 (20)

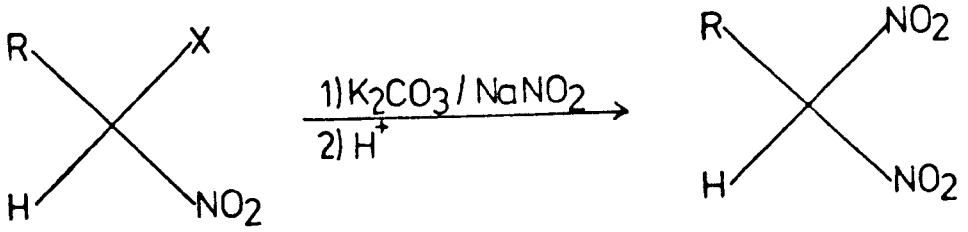
(ii) With n-Butyl Lithium in DMSO

Ketal bromonitroamide (50.9 mg, 0.1 mM) was flushed with argon for 30 minutes, then dissolved in dry DMSO (2.5 ml). n-Butyl lithium (82 µl of a 1.35 M solution in hexane, 0.11 mM) was added via a rubber septum and the solution was stirred at room temperature and irradiated with a 150W tungsten lamp for 2 hours. Ice-water was added and the solution neutralised with 1N HCl. The solution was extracted with Et_2O, followed by washing, drying and evaporation to yield a pale yellow oil which would not crystallise. The course of the reaction was followed by removing aliquots, working up as above and examining by t.l.c. (Sigel; 2:3 EtOAc / benzene; and 2% MeOH / CH_2Cl_2) which indicated several products.

n.m.r. ($CDCl_3$) : δ = 1.28 (t, 7H), 1.4 - 2.2 (m, 14H), 3.90 (S, 4H), 4.25 (q, 7, 2H), 5.05 (m, q?, 1H), No NH observable

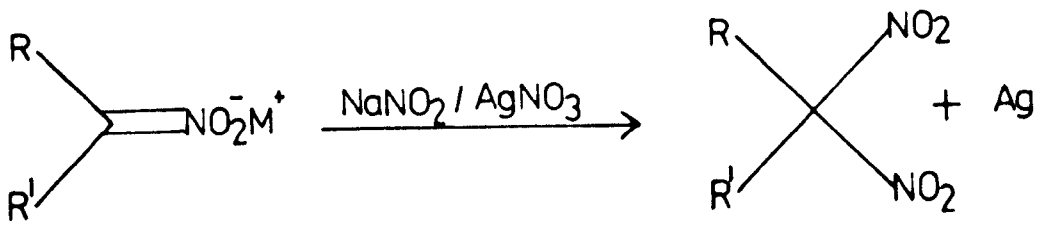
i.r. (CH_2Cl_2) : νcm^{-1} 3410 m, 2940 m, 2890 m, 1745 br s, 1685 s, 1605 m, 1550, 1370 w, 1190 w, 1100 w, 1025 m, 950 w

CHAPTER II

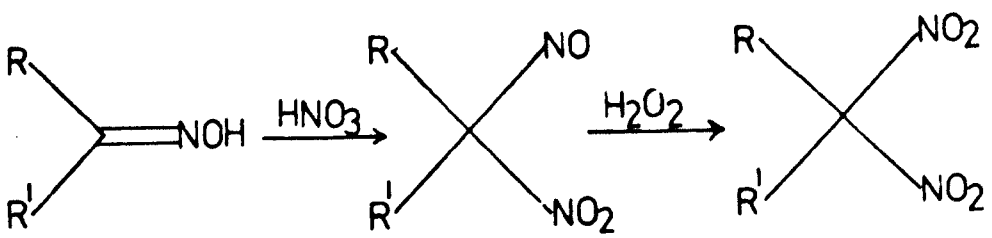


X= Halogen

SCHEME I



SCHEME II



SCHEME III

Reactions of Polynitro Compounds

Secondary nitronate anions undergo radical-anion coupling reactions with geminal dinitro compounds[21] *via* the proposed[21] mechanism described in Chapter 1 (Scheme I). During the investigations concerning the reactions of primary nitronate anions with gem halogenonitro compounds, a simultaneous study using gem dinitro compounds was carried out. It was envisaged that this approach might be an alternative to a bromonitro coupling, in the preparation of the A/D component. Reactions of primary nitronate anions with gem dinitro compounds are discussed in this chapter for reasons of collocation.

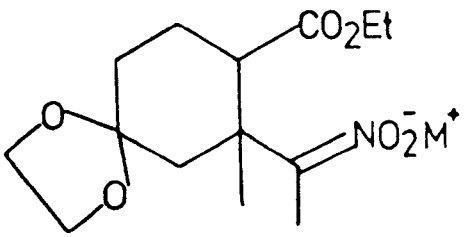
Although secondary nitronate anions react with gem dinitro compounds to give highly substituted coupled products, the limitations of the applicability to hindered systems had not been established. Modified ring A precursors and geminal dinitro esters were used to determine the synthetic generality of this reaction. Also, a ring A precursor was prepared by which this reaction could be applied in an intramolecular manner, to join available ring A and ring D intermediates.

Synthesis of gem Dinitro Compounds

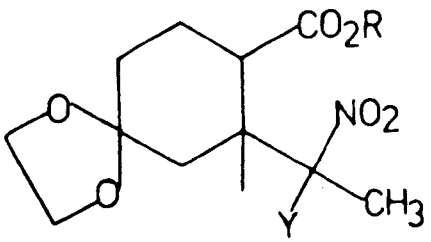
Several methods for preparing geminal dinitro compounds have been described and reviewed.[62] The ter Meer reaction[63] and the Kaplan-Shechter oxidative nitration[64] probably have the widest synthetic application. The former reaction (Scheme I) is only useful for preparation of secondary dinitro compounds. Shechter's method (Scheme II) however, is effective using salts of primary or secondary nitro compounds and may be used for hindered compounds. The reaction is unsuccessful for nitro compounds which have an electron withdrawing group alpha to the carbon bearing the nitro group (e.g. where R' =-CO_2R,-CN, -$CONR_2$ or-COR). A further method is through nitration of oximes to pseudonitroles, which are easily oxidised[65] to the corresponding gem dinitro compound.

Coupling Reactions in Simple Model Systems

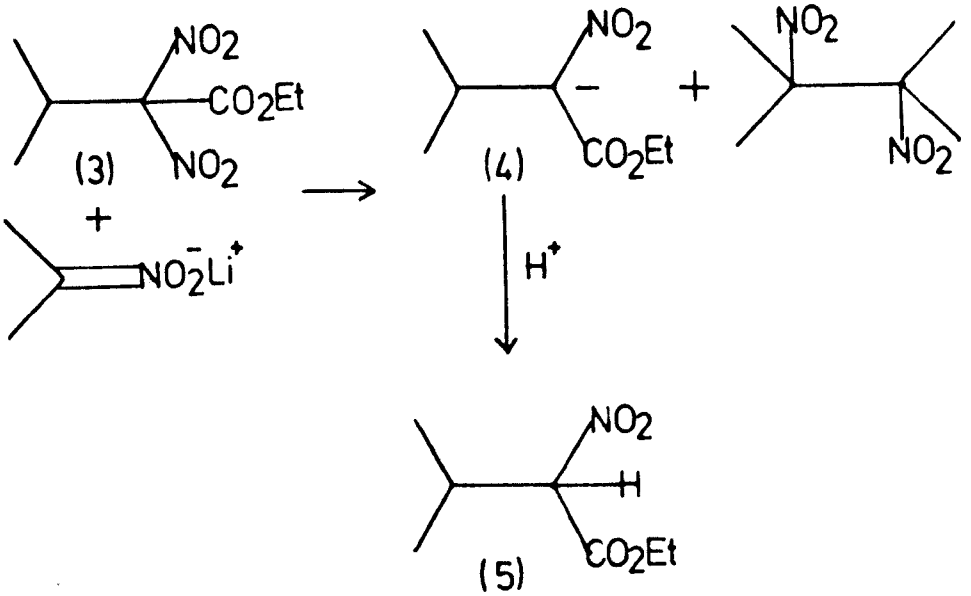
Literature methods were used for the preparation of model geminal dinitro compounds. 2,2-Dinitropropane and 1,1-dinitrocyclohexane were prepared by oxidative nitration of the corresponding nitro compounds. 2,2-Dimethyl-3-nitrobutane was not available in good yield by the Iffland procedure[56] so other routes to 2,2-dimethyl-3,3-dinitrobutane were examined. This compound could be


CO2Et
O
O
NO2⁻M⁺

(1)


CO2R
O
O
NO2
Y
CH3

(2)


NO2
CO2Et
NO2
(3)
+
NO2⁻Li⁺
NO2
CO2Et
(4)
−
+
NO2
NO2
H⁺
NO2
H
CO2Et
(5)

prepared by nitration of pinacolone oxime with nitric acid and subsequent oxidation of the pseudonitrole, but the yield was very low.

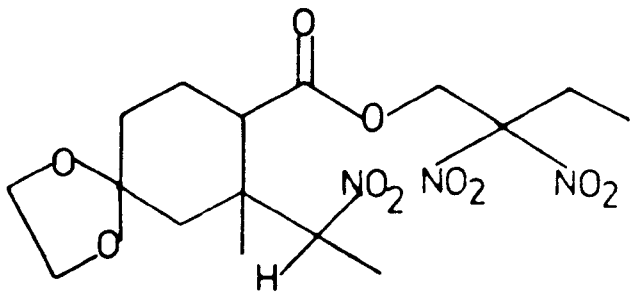
Reactions of these model dinitro compounds were studied under similar conditions to those used for attempted couplings with bromonitro models (Chapter 1). 2,2-Dinitropropane was converted by treatment with thallium 1-propanenitronate to a mixture of 2-methyl-2,3-dinitropentane, 2-methyl-3-nitropent-2-ene and 2,3-dimethyl-2,3-dinitrobutane in amounts dependent on the temperature and duration of the reaction. Attempts to obtain 2-methyl-2,3-dinitropentane in a pure form from this reaction were unsuccessful as, again, it proved impossible to separate this material from the nitro-olefin. There was substantially no reaction between TPN and 2,2-dimethyl-3,3-dinitrobutane, although absorptions corresponding to those exhibited by pinacolone were present in the infrared spectrum of the crude reaction material.

Lithium 1-propanenitronate reacted with 2,2-dinitropropane to give a mixture of products which again included 2-methyl-2,3-dinitropentane, 2-methyl-3-nitropent-2-ene and 2,3-dimethyl-2,3-dinitrobutane. Reaction of 2,2-dimethyl-3,3-dinitrobutane with lithium 1-propanenitronate also gave a complex mixture of products and it was not clear from the spectroscopic data whether coupling had occurred or not. The dinitro starting material could not be prepared in sufficient quantity for this reaction to be studied in great detail.

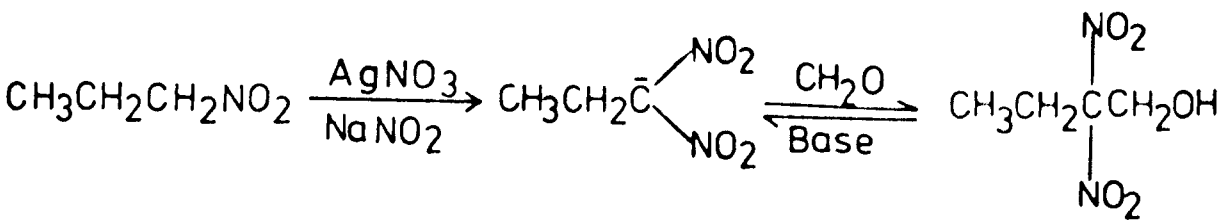
It became increasingly apparent that coupling reactions using a primary nitronate anion were unlikely to be preparatively useful. As a precaution, in order to confirm that Kornblum's experiments[21] were reproducible, 1,1-dinitrocyclohexane was reacted with lithium 2-propanenitronate in DMSO. Reasurringly an 84% conversion to 1-nitro-1-(2'-methyl-2'-nitroethyl)cyclohexane occurred.

Geminal Dinitro Esters

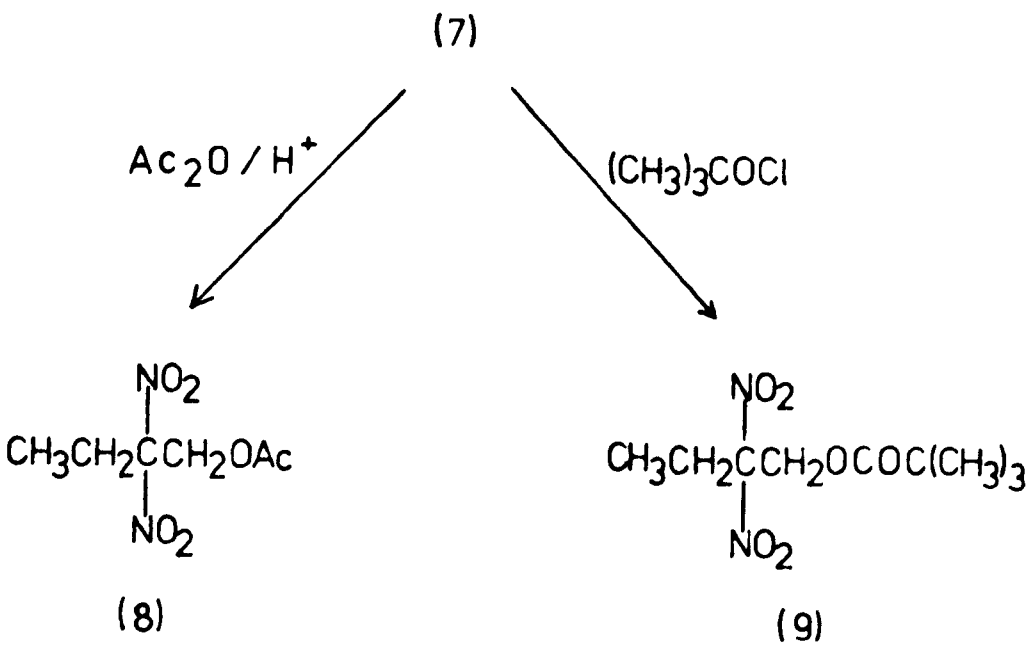
After the failures with primary nitronate anions, attention was turned to the possible use of a secondary nitronate anion in a ring A to ring D coupling reaction. This meant that ring A precursor (1) would be the secondary nitronate anion. As a consequence, a dinitro ring D precursor would require protection of the acidic hydrogen at the carbon carrying the two nitrogroups. The reaction between a secondary nitronate anion and a dinitro ester of type (3) was a possibility which could be incorporated into the synthesis of the A/D component. However,



(6)



(7)



(8) (9)

reaction of lithium 2-propanenitronate with ethyl 2,2-dinitro-3-methylbutyrate (3) caused rapid conversion to 2,3-dimethyl-2,3-dinitrobutane and the nitronate anion (4). Subsequent protonation on work up gave ethyl 2-nitro-3-methylbutyrate (5). Here n.m.r. measurements indicated that nitrogroup abstraction is a fast reaction and the resulting 2,2-dinitropropane then reacts with unreacted 2-propanenitronate anion. It was evident that compounds of type (3) were unlikely to be of synthetic use in coupling reactions.

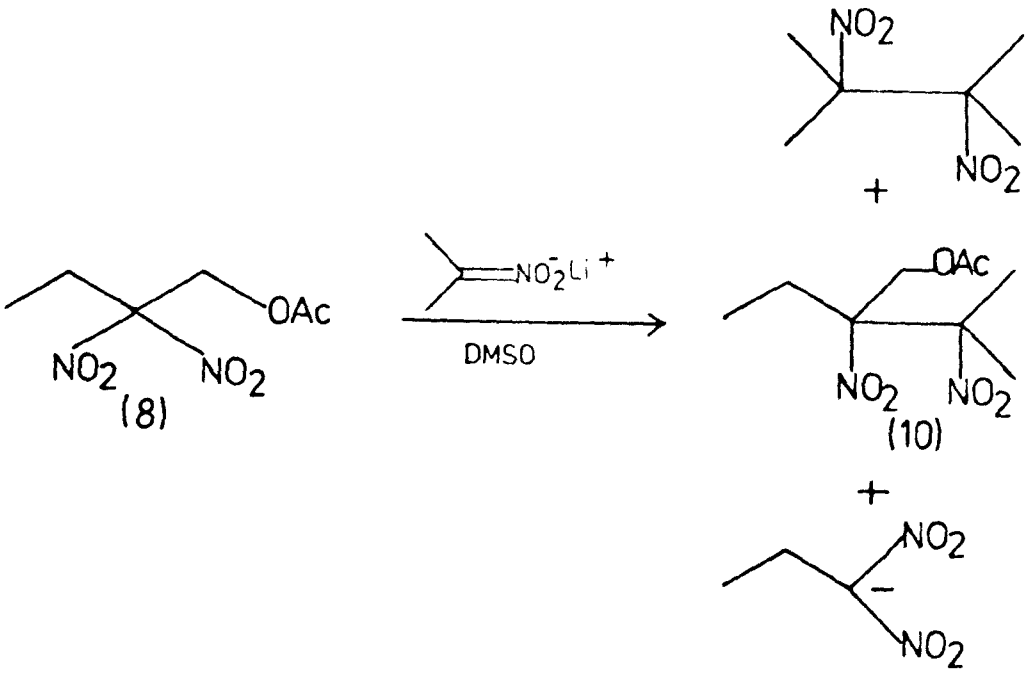
The steric hindrance at the nitroethyl group in compounds of type (2) had been observed in studies with bromonitro compounds. It came as no surprise then when an intermolecular coupling between the lithium nitronate salt (1) and 2,2-dinitropropane was unsuccessful. Monitoring the reaction by ^{1}H n.m.r. in $^{2}H_{6}$-DMSO indicated that acetone was a product of the reaction. On a preparative scale, however, only unreacted 2,2-dinitropropane and starting material were recovered. In ethanol, no coupled products were observed after 6 days reaction, although by this time the anion appeared to be decomposing. This offered further evidence that an intermolecular coupling via a nitro-coupling reaction with the available precursors was likely to be impossible.

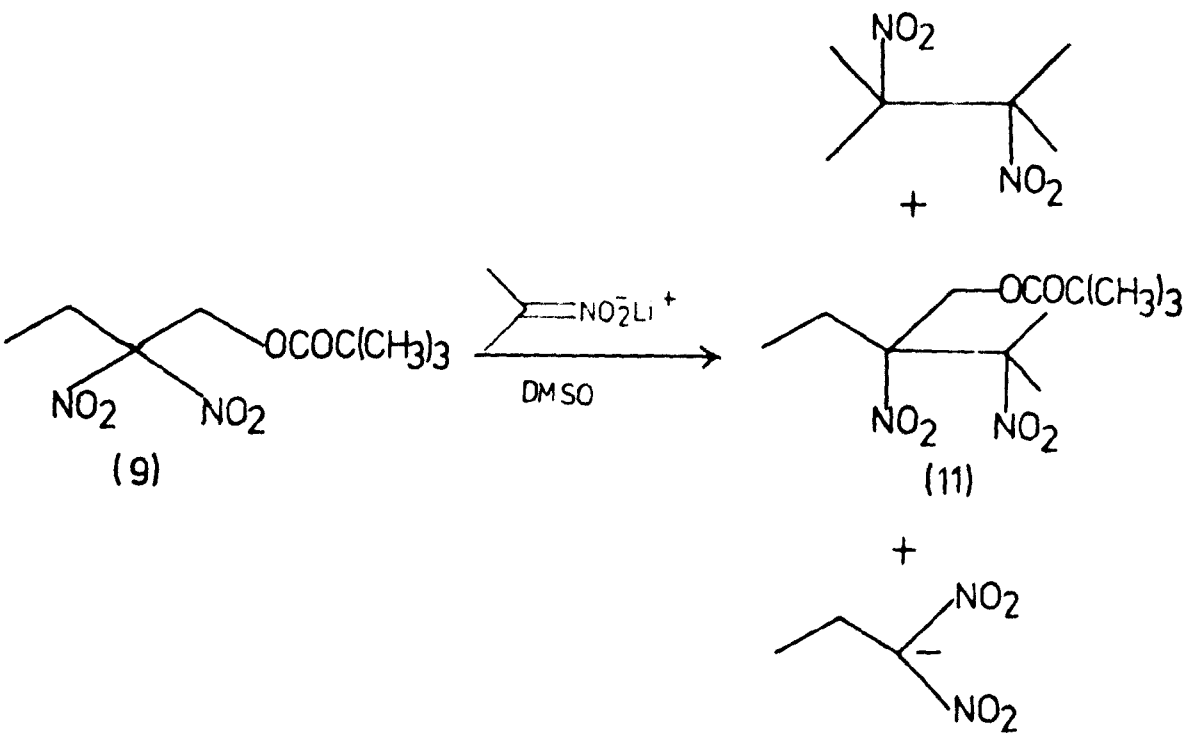
There was, of course, the last possibility of incorporating the nitronate anion and dinitro centre into the same molecule so that intramolecular cyclisation could be examined. The trinitro ester (6) was the projected precursor and if cyclisation occurred, then the model could be easily modified to incorporate the ring D precursor, thus making maximum use of available intermediates. Firstly, however, some simple model compounds were synthesised and their properties examined.

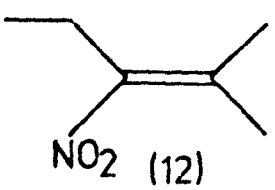
Simple Dinitroalkyl Esters

Shechter's oxidative nitration had been modified[66] to make β-dinitro-alcohols available from nitroalkanes,and 2,2-dinitro-1-butanol (7) was readily prepared by this method. β-Dinitro alcohols under basic conditions and in polar solvents dissociate[67] to formaldehyde and the corresponding nitronitronate anion.

(7) could be quantitatively acetylated to 1-acetoxy-2,2-dinitrobutane (8) by acetic anhydride and a catalytic amount of sulphuric acid. Reacting the dinitro-alcohol with pivaloyl chloride gave the t-butyl analogue (9). These two esters were used for model experiments. The dinitro ester (8) reacted with lithium 2-propane-


NO2
NO2
+
OAc
NO2
NO2
(8)
NO2Li+
DMSO
OAc
NO2
NO2
(10)
+
NO2
NO2


NO2
NO2
+
OCOC(CH3)3
NO2
NO2
(9)
NO2Li+
DMSO
OCOC(CH3)3
NO2
NO2
(11)
+
NO2
NO2


NO2
(12)

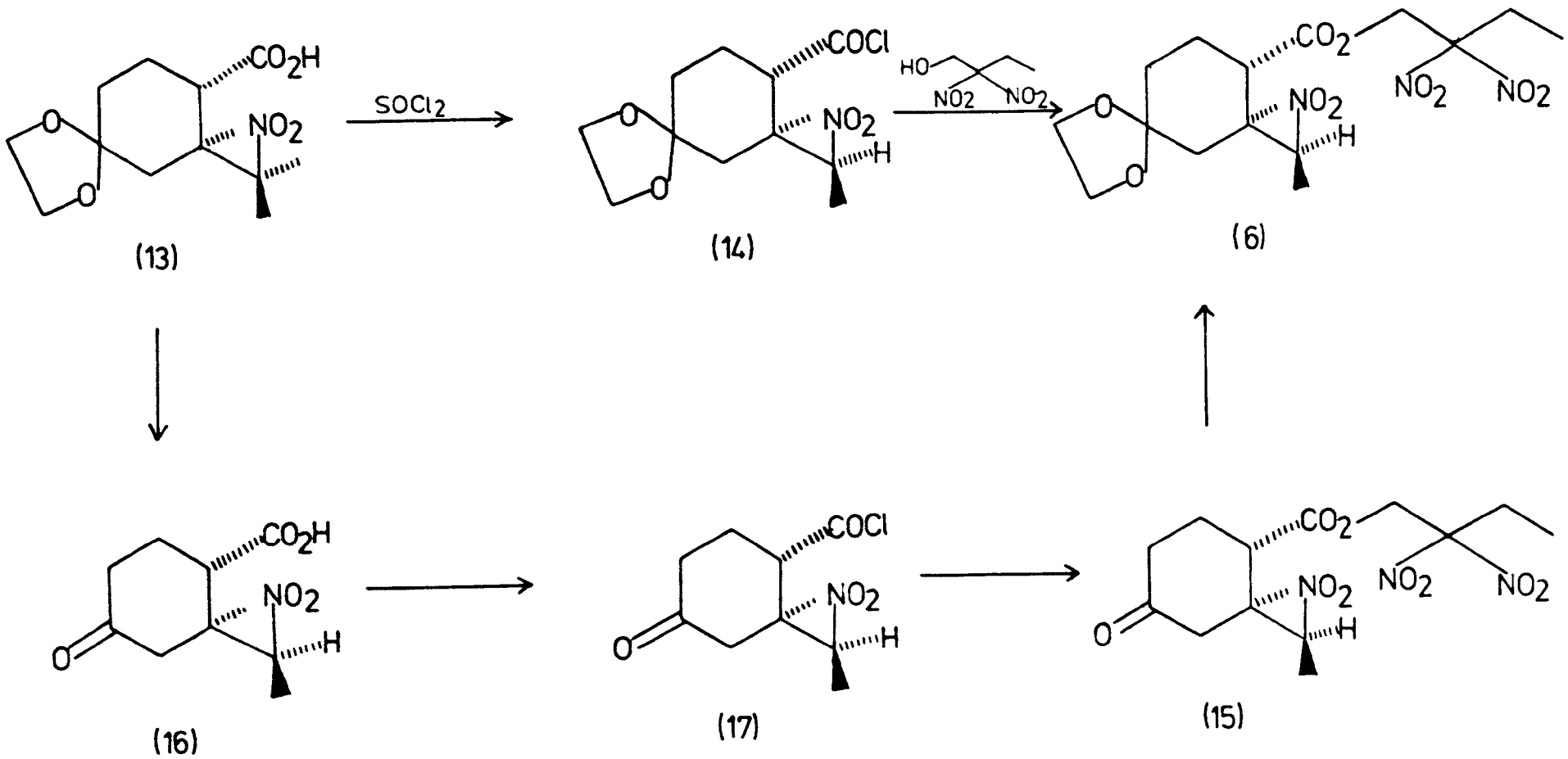
nitronate yielding the highly substituted coupled compound (10). The ubiquitous 2,3-dimethyl-2,3-dinitrobutane was also isolated together with 1,1-dinitropropane which arose from protonation of its anion on work up. The former byproduct arose due to nitrogroup abstraction by the nitronate anion followed by coupling with unreacted anion as noted earlier. The 1-nitro-1-propanenitronate anion presumably arose due to attack at the carbonyl carbon of the ester. Attempts to minimise this side reaction, for instance, by use of a smaller excess of nitronate salt, or by inverse mixing of the reactants (i.e. adding a solution of the nitronate salt to the dinitro compound) did not improve the yield of (10). In the t-butyl analogue (9) greater steric hindrance at the carbonyl carbon would be expected. Reacting (9) with lithium 2-propanenitronate gave the corresponding coupled compound (11) but, again, 1-nitropropanenitronate anion was formed. Subsequently it was found that the coupling reaction was particularly dependent on the solvent. For example, no reaction occurred between lithium 2-propanenitronate and (8) in dioxan, whereas in ethanol, a mixture of products resulted from which 2,3-dimethyl-2,3-dinitrobutane and 1,1-dinitropropane could be identified. There was no coupled product (10) in the mixture.

Notwithstanding these drawbacks, this approach allowed these facile substitution reactions to be applied to starting materials such as 1,1-dinitropropane which had acidic hydrogen atoms. The protecting group could be removed after a successful coupling had occurred. It was also envisaged that these vicinal dinitro esters could perhaps be used in the synthesis of nitro-olefins. Literature methods for nitro-olefin synthesis rely on the condensation of a nitronate anion and a ketone to give an α-nitroalcohol which is subsequently dehydrated.[68,69] These methods frequently result in low yields when applied to all but the simplest systems,[70] so any new general nitro-olefin synthesis would be of some interest.

Treatment of (10) with sodium methoxide in methanol was anticipated to lead to 2-methyl-3-nitro-pent-2-ene (12). However, the reaction did not appear to give a straightforward conversion and the products have not been completely characterised. It is yet to be established whether the course of the reaction is strongly susceptible to the reaction conditions.

Synthesis of a Trinitro Ester for Possible Intramolecular Cyclisation

The success of the coupling reaction nonetheless was encouraging, so


CO2H
NO2
SOCl2
COCl
NO2
H
HO
NO2
NO2
CO2
NO2
NO2
NO2
H
O
O
(13)
(14)
(6)
CO2H
NO2
H
O
COCl
NO2
H
O
CO2
NO2
NO2
NO2
H
O
(16)
(17)
(15)

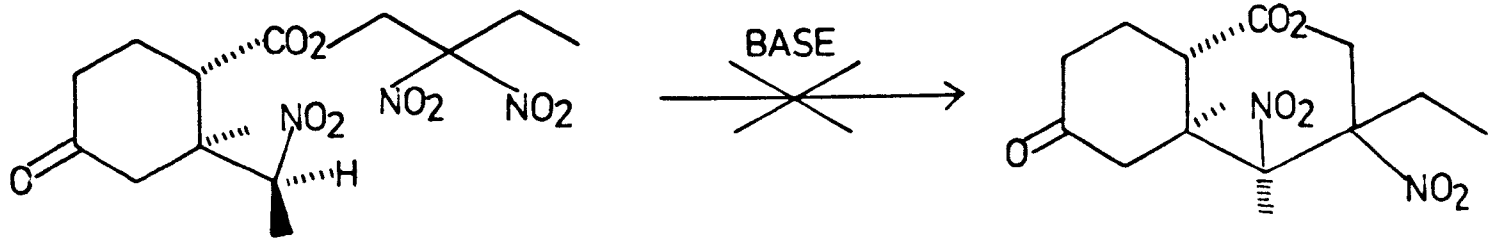
SCHEME IV

efforts were directed to the synthesis of the ring A precursor (6) to examine whether it could be intramolecularly cyclised under basic conditions. The synthesis of (6) by a seemingly trivial route (Scheme IV) proved notably difficult. The ketal nitro acid (13) was easily converted to the acid chloride (14) by thionyl chloride in benzene. Literature methods[71] were available for the conversion of 2,2-dinitro-1-alkanols to their esters by reaction with equimolar quantities of acid chloride and pyridine in dichloromethane. When applied to our model compounds, this method was not successful, since the pyridine caused dissociation of the 2,2-dinitrobutanol and the resulting nitronate anion was O-acylated by the acid chloride giving a nitronic anhydride. Some normal esterification also occurred and mixtures of products ensued. More hindered bases such as di-isopropylethylamine also caused formation of the nitronic anhydride which was not isolated but whose presence was inferred from the i.r. spectrum of the crude reaction mixture (strong C=O absorption at 1810 cm^{-1}). Activation of the acid chloride with silver tetrafluoroborate was only partially successful in helping esterification, and deketalisation also occurred during reaction with the alcohol. Direct reaction of the acid chloride and alcohol was slow in solution, and fusion of the reactants was the only method which led to complete reaction. When a base was not present to neutralise the HCl formed, then the ethylene ketal in (6) was partially liberated, giving a mixture of ethylene ketal (6) and the keto compound (15) which were tedious to separate. Neutral acid scavengers such as magnesium powder were not helpful; presumably magnesium chloride also catalysed deketalisation. Only partial esterification occurred in an acid-catalysed reaction between the ketal nitro acid (13) and the dinitro alcohol. Hence, the approach was altered such that the ketal function was removed quantitatively in a step prior to esterification. The ester (15) was then obtained in reasonable yield by heating the acid chloride (17) directly with 2,2-dinitrobutanol. The majority of the cyclisation studies were carried out on this compound rather than the ethylene ketal (6).

Attempted Cyclisation Reactions

Reaction of (6) with $NaOCD_3$

In an attempt to form the nitronate anion of (6) using sodium $^2H_3$-methoxide in $^2H_4$-methanol, the base rapidly attacked the ester carbonyl, liberating 1-nitro-1-propanenitronate anion, which was apparent from its characteristic yellow colour.

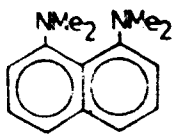


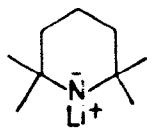
(15)

BASES:

NaH

$NaNH_2$





On work up, 1,1-dinitropropane was identified. The lability of the ester group was recognised as a serious drawback to cyclisation. If the nitronate anion could be formed at the nitroethyl centre, then it was possible that it would also attack the ester carbonyl.

Attempted Cyclisation of (15)

Because of the sensitivity of the ester carbonyl to nucleophilic bases, some alternatives were examined. Sodium hydride is unreactive towards esters, so was mixed with the ester in dry $^{2}H_{6}$-DMSO and the deprotonation observed by ^{1}H n.m.r. Deprotonation was very slow and there were no signs of cyclisation after 6 hours, by which time signals suggesting the presence of 2,2-dinitrobutanol were observed in the spectrum. On work up a mixture of many products resulted. None were identified as cyclised material, although no 1,1-dinitropropane was isolated. It was possible that here the dimsyl anion may have formed and caused some decomposition of the ester.

Sodium amide is frequently a useful alternative to sodium ethoxide. At room temperature or at 100° C in dioxan, sodium amide did not deprotonate the nitromethine carbon in (15). After heating at 100° C in DMSO, extensive decomposition occurred leading to a complex mixture of products. The problem lay in specific removal of the nitromethine proton, so that the nitronate anion could be generated quantitatively, and in a suitable solvent where it might intramolecularly react with the dinitro side chain.

The synthetic values of bases capable of abstracting protons from substrates containing sites particularly reactive towards nucleophilic attack, has been recognised. The more successful reagents have achieved distinctive names - for example, 'Proton Sponge' (1,8-bis(dimethylamino)naphthalene)[72]. The pKa of proton sponge is 12.34 which is higher than normal aliphatic amines. However, on reacting proton sponge with the ester (15) in $^{2}H_{6}$-DMSO, no deprotonation occurred at room temperature. Warming the reactants at 95° - 100° C was necessary to initiate reaction and then it appeared that epimerisation at the nitromethine carbon was occurring rather than complete deprotonation. After long periods (3 days) of warming and irradiation, no cyclised product could be observed. Although deprotonation was obviously occurring, the low concentration of nitronate anion was insufficient for effective cyclisation.

Olofson has recently reported[73] the use of lithium 2,2,6,6-tetramethylpiperidide (Li TMP) as a proton-specific base. It is easily prepared by treating 2,2,6,6-tetramethylpiperidine with n-butyl lithium in an inert solvent. The choice of solvent for an attempted cyclisation reaction was particularly limited. DMSO is the preferred solvent for 'Kornblum-type' couplings, but preparation of Li TMP in DMSO would also result in the formation of some lithium dimsyl. Indications from experiments using sodium hydride in DMSO suggested that the ester (15) was attacked by dimsyl anion. In dioxan, Li TMP did not deprotonate the nitro carbon in (15) after 16 hours and only unreacted starting material was recovered. Dioxan was not a favourable solvent for coupling reactions as in this solvent lithium 2-propanenitronate fails to react with the dinitro ester (8). Hexamethylphosphoramide (HMPA) is stable to strong bases so was an obvious combination to examine with Li TMP as dipolar aprotic solvents are the most effective for coupling reactions. ^{1}H n.m.r. indicated that Li TMP instantaneously deprotonated the ester (15) when dissolved in HMPA. There was no indication from the n.m.r. in situ that cyclisation had occurred. On work up a low yield of an oily product, which had a badly resolved n.m.r. spectrum, resulted. It could not be identified from its spectral data, but the i.r. spectrum showed two NO_2 stretching frequencies at 1573 and 1550 cm^{-1}. The coupled product would not be expected to exhibit this behaviour by comparison with data for simple models such as (10) or (11). Also 1,1-dinitropropane was identified as a product in the reaction mixture. The inherent lability of this precursor (15) towards bases strongly suggested that even if modified by incorporation of a ring D precursor, it was unlikely that a successful cyclisation could be developed.

After these disappointing setbacks it was felt that this approach was strongly unfavourable and work with this precursor was discontinued.

EXPERIMENTAL II

Simple Nitronate Salts

The preparation of nitronate salts used is described in Chapter 1.

Simple Geminal Dinitro Compounds

2,2-dinitropropane

Shechter's oxidative nitration was used.[64] Aqueous NaOH (9.05 g, 0.226 M in 20 ml) was added dropwise to a mixture of 2-nitropropane (17.8 g, 0.2 M) in water (45 ml), cooled to 0 - 10° C, followed by addition of $NaNO_2$ (15.6 g, 0.226 M) in water. The mixture was stirred for 10 minutes at 0 - 10° C, then quickly added to a vigorously stirred solution of aqueous silver nitrate (68.3 g 0.41 M in 100 ml) at 0° C. The cream coloured suspension that initially formed turned black and the reaction mixture was allowed to warm to room temperature. Stirring was continued for 20 minutes, then the silver was filtered off on to Celite. The filtrate was extracted with ether-benzene and extracts washed with brine. Drying and evaporation yielded a colourless oil which was distilled under reduced pressure (70° / 0.5 mm). The colourless distillate (13 g, 50%) crystallised on cooling.

m.p. : 53° (lit. 53°)[74]

n.m.r. (CCl_4) : δ = 2.15 (S)

2,2-dimethyl-3,3-dinitrobutane

Anhydrous HNO_3 was prepared for the nitration. A mixture of 98% H_2SO_4 (200 ml) and 70% HNO_3 (200 ml) was distilled under reduced pressure (~40° C / 15 mm) collecting the first 100 ml of distillate. The anhydrous HNO_3 was redistilled before each usage and urea (100 mg) added per 10 ml of acid in order to remove nitrites.[65] The oxidation is potentially dangerous and suitable safety precautions were taken.

Anhydrous HNO_3 (20 ml) was added cautiously during 75 minutes to a solution of pinacolone oxime (5.0 g, 43 mM) in dry Et_2O (50 ml), maintaining the temperature at - 5° — 0° C. An intense blue colour developed during the addition. Stirring was continued a further 20 minutes at - 5° C, then allowed to warm to 15° C. Hydrogen peroxide (10 ml, 100 vol) was cautiously added, maintaining the temperature at 15° - 20° C and the mixture stirred until the blue colour had completely disappeared (ca. 3 hours). The mixture was then diluted with water and extracted with Et_2O. Extracts were washed with aqueous $NaHCO_3$, aqueous $FeSO_4$ and water, then dried. Evaporation gave a pale green solid (1.3 g) which was dissolved in pentane and

chromatographed on silica gel (50 g). Eluting with 1 : 1 CH_2Cl_2 / pentane then CH_2Cl_2, 2,2-dimethyl3,3-dinitrobutane (740 mg, 10%) was contained in the first fractions. Further purification was by recrystallisation from ethanol and then sublimation at atmospheric pressure. The product was a white camphorous-smelling solid, which was very volatile if stored under reduced pressure.

m.p.	:	174 - 175° C (subl., sealed tube) lit. 173 - 174° C[75]
n.m.r. ($CDCl_3$)	:	δ = 1.31 (S, 9H), 2.16 (S, 3H)
i.r. (KBr)	:	νcm^{-1} 2990 m, 2960 m, 2890 m, 1580 - 1550 s

1,1-Dinitrocyclohexane

Using Iffland's procedure[56] cyclohexanone oxime was converted to nitro-cyclohexane which was oxidatively nitrated to 1,1-dinitrocyclohexane by the method described for 2,2-dinitropropane. The product was a colourless oil (89% yield from nitrocyclohexane) which crystallised on storage.

m.p.	:	25 - 27° C
n.m.r. ($CDCl_3$)	:	δ = 1.53 - 1.9 (m, 6H), 2.45 - 2.8 (m, 4H)
i.r. (liq.film)	:	νcm^{-1} 2950 m s, 2870 m s, 1580 - 1540 s
n_D^{27}	:	1.4856 (lit. n^{21} 1.4732)[76]

Reactions of Dinitro Compounds with Thallium 1-Propanenitronate

With 2,2-dinitropropane

The general method used was as that described for similar reactions with 2,2-bromonitropropane (Chapter 1, page 29). The effects of temperature and length of reaction were studied in separate experiments. Consistent results were difficult to obtain although, in general, 2-methyl-2,3-dinitropentane was always the initial product, and as reaction proceeded, 2-methyl-3-nitropent-2-ene predominated. Reactions never progressed to completion and 2,2-dinitropropane was always present in the reaction mixture, even after using an excess of the thallium salt.

Attempted Preparation of 2-methyl-2,3-dinitropentane

Several attempts were made, the following is typical :

2,2-Dinitropropane (670 mg, 5 mM) in dry MeCN (10 ml) was added to TPN (2.55 g, 8.75 mM) suspended in MeCN (40 ml) under N_2. The suspension was irradiated with stirring for 5 hours, then worked up as usual to yield a yellow oil

(630 mg). ^{1}H n.m.r. indicated that 2,2-dinitropropane, 2-methyl-2,3-dinitropentane and 2-methyl-3-nitropent-2-ene were present in the ratio 3.2:2.5:1.0. In another smaller scale experiment the ratio was 2.0:4.6:1.0 respectively. Consequently it was difficult to determine the optimum time of reaction in order to maximise the yield of the vicinal dinitro compound. The nitro-olefin and vicinal dinitro compound were extremely difficult to separate as noted before. Separation of 2,2-dinitropropane could be achieved by g.l.c. (2' S.E.30, 100° C) but the vicinal dinitro compound decomposed to the nitro-olefin and was never isolated pure.

With 2,2-dimethyl-3,3-dinitrobutane

An identical procedure to that used for the reaction of 2,2-bromonitro-3,3-dimethylbutane with TPN was employed. After 10 hours there was substantially no reaction although weak absorptions due to pinacolone could be seen in the i.r. spectrum of the crude reaction product.

Reactions with Lithium 1-Propanenitronate

With 2,2-dimethyl-3,3-dinitrobutane

Lithium 1-propanenitronate (166 mg, 1.73 mM) was reacted with the dinitro compound (176 mg, 1 mM) in dry DMSO in the usual way under N_2. The solution became very dark brown after a few minutes reaction, and no starting material could be detected by t.l.c. after 30 minutes. After work up, a brown oil (78 mg) which crystallised was isolated and recrystallised with some difficulty from EtOH (-78° C). This compound was not the expected coupled product since no ethyl group could be observed in the ^{1}H n.m.r. spectrum, but its identity was not established.

With 2,2-dinitrobutane

The above procedure was employed on a 1 mM scale and, again, a brown solution resulted after several minutes. On work up, a yellow oil (104 mg) was isolated. ^{1}H n.m.r. indicated a mixture of 2-methyl-2,3-dinitropentane, 2-methyl-3-nitropent-2-ene and 2,3-dimethyl-2,3-dinitrobutane which were not separated.

Reaction of Lithium 2-Propanenitronate with 1,1-Dinitrocyclohexane

Lithium 2-propanenitronate (329 mg, 3.46 mM) was flushed with argon for 30 minutes and then dissolved in dry DMSO (10 ml). 1,1-Dinitrocyclohexane (348 mg, 2 mM) in DMSO (10 ml) was added *via* a rubber septum and the reaction stirred and irradiated at room temperature. After 90 minutes there was no starting material left. Addition of ice water followed by extraction with Et_2O

afforded crystalline 1-nitro-1-(2'-methyl-2-nitroethyl)cyclohexane (368 mg, 84%) which was one component by t.l.c. (Sigel ; CH_2Cl_2). Recrystallisation was from EtOH to yield white platelets.

m.p. : 145° C (lit.[77] 139° C)

n.m.r. ($CDCl_3$) : δ = 1.20 - 1.85 (m, 6H), 1.68 (S, 6H), 2.45 - 2.70 (m, 4H)

i.r. (CH_2Cl_2) : νcm^{-1} 2960 m s, 2870 m, 1545 s

Preparation of Simple α,α-Dinitro Esters

α,α-Dinitro esters are prepared[78] in low yield by nitration of half esters of malonic acid and alkyl malonic acids with 70% nitric acid. Monoethyl isopropyl malonate was prepared by a modification[78] of the method of Marguery,[79] and was used directly as isolated, without further purification.

n.m.r. ($CDCl_3$) : δ = 0.97 (d, 7, 3H), 1.00 (d, 7, 3H), 1.25 (t, 7, 3H), 2.20 (m, 1H), 3.10 (d, 9, 1H), 4.15 (q, 7, 2H), 12.50 (S, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3475 br w, 2935 m, 2880 m, 1750 - 1700 s

Ethyl-1,1-dinitro-2-methylbutyrate (3)

70% HNO_3 (14 ml) was added dropwise to monoethyl isopropyl malonate (2.0 g, 11.7 mM), warmed to 50° C. The mixture became green, evolving CO_2 and nitrous fumes which were removed by suction. Stirring at 50° C was continued for 1 hour and then the mixture was diluted with ice water and extracted with CH_2Cl_2. The organic extracts were washed with aqueous $NaHCO_3$ until there was no further effervescence, then water and dried. Evaporation gave a blue oil (625 mg). (Note : omission of the basic wash at the end of the reaction yields a product considerably contaminated with acidic side products). The crude product was distilled under reduced pressure to give a colourless distillate (b.p. 79 - 81° C/ 0.5 mm) (300 mg, 13%).

n.m.r. ($CDCl_3$) : δ = 1.25 (d, 7, 3H), 1.33 (t, 7, 3H), 3.12 (m, 1H), 4.40 (q, 7, 2H)

i.r. (liq.film) : νcm^{-1} 2985 m s, 2945 m, 2900 m, 1760 s, 1735 sh, 1575 s

n_D^{27} : 1.4345

		% C	H	N
$C_7H_{12}N_2O_6$	requires :	38.18	5.49	12.72
	found :	38.29	5.55	12.63

Preparation of Simple 2,2-Dinitroalkyl Esters

2,2-Dinitrobutanol (7)

Preparation was by a method described for 2,2-dinitropropanol.[66] Aqeuous NaOH (16.8 g, 0.42 M in 60 ml) was added during 10 minutes to a vigorously stirred suspension of 1-nitropropane (33.10 g, 0.372 M) in water (40 ml) cooled to 0 - 10° C. Stirring was continued until the solution was homogeneous, then an aqeuous solution of $NaNO_2$ (28.8 g, 0.42 M in 40 ml) was added. The solution was stirred for 10 minutes at 0 - 10° C, then quickly added to a vigorously stirred solution of silver nitrate (128.8 g, 0.76 M) in water (480 ml) at 0° C causing the temperature to rise to 15° C. After stirring for an additional 30 minutes, the mixture was treated with 50% aqueous NaOH until the pH was ca. 12. The suspension was filtered and the silver washed with water until the washings were colourless. The pH of the combined filtrates was adjusted to 8.5 - 9.5 by addition of dilute acetic acid, then formaldehyde (34.0 g of a 37% solution in water, 0.42 M) was added quickly. The solution was stirred for 15 minutes, then dilute acetic acid was added to adjust the pH to 5. The solution was extracted well with CH_2Cl_2; drying and evaporation gave 2,2-dinitrobutanol (47.8 g, 78%) which was one component by t.l.c. The crude material was distilled in vacuo collecting a mid-fraction b.p. 65 - 69° C / 0.1 mm (40.2 g, 66%).

n.m.r. ($CDCl_3$) : δ = 1.05 (t, 7, 3H), 2.59 (q, 7, 2H), 2.94 (t, 7, 1H; exchanges with D_2O), 4.40 (d, 7, 2H; collapses to S with D_2O)

i.r. (liq.film) : νcm^{-1} 3560 m, 3430 br m, 2980 m, 2950 m, 2890 m, 1580 - 1560 vs

n_D^{27} : 1.4575

		% C	H	N
$C_4H_8N_2O_5$	requires :	29.27	4.91	17.07
	found :	29.52	5.06	17.10

Preparation of 1-Acetoxy-2,2-Dinitrobutane (8)

One drop of 98% H_2SO_4 was added to a vigorously stirred mixture of 2,2-dinitrobutanol (8.2 g, 50 mM) and acetic anhydride (5.62 g, 50 mM) at room temperature. The reaction was exothermic and the temperature rose to ca. 60° C. The mixture was allowed to cool with stirring for 30 minutes. The product was washed with aqueous $NaHCO_3$, then water, and extracted with Et_2O. Drying and

evaporation gave a pale yellow oil (9.5 g, 92%) which was pure by n.m.r. The product was distilled at reduced pressure (b.p. 60 - 62° C / 0.1 mm).

n.m.r. ($CDCl_3$) : δ = 1.04 (t, 7, 3H), 2.06 (S, 3H), 2.58 (q, 7, 2H), 4.95 (S, 2H)

i.r. (liq.film) : νcm^{-1} 2980 w, 2950 w, 2890 w, 1765 s, 1580 - 1560 vs

n_D^{27} : 1.4408

		% C	H	N
$C_6H_{10}N_2O_6$	requires :	34.95	4.89	13.59
	found :	35.16	4.65	13.52

Preparation of 2,2-Dinitro-1-pivaloyloxybutane (9)

A mixture of 2,2-dinitrobutanol (8.2 g, 50 mM) and pivaloyl chloride (7.2 g, 60 mM) were refluxed in dry benzene (5 ml) for 1 hour, after which no alcohol was left. The solvent and unreacted pivaloyl chloride were removed in vacuo to give a quantitative yield of the pivaloate (12.35 g, 99%) which was pure by n.m.r. The colourless oil was distilled at reduced pressure (b.p. 75° C/ 0.1 mm).

n.m.r. ($CDCl_3$) : δ = 1.05 (t, 7, 3H), 1.18 (S, 9H), 2.58 (q, 7, 2H), 4.9 (S, 2H)

i.r. (liq.film) : νcm^{-1} 2975 m s, 2880 m, 1750 s, 1570 vs, 1560 vs

n_D^{27} : 1.4378

		% C	H	N
$C_9H_{16}N_2O_6$	requires:	43.54	6.50	11.29
	found:	43.21	6.38	11.45

Reactions of Lithium 2-Propanenitronate with Gem Dinitro Esters.

With Ethyl-1,1-Dinitro-2-methylbutyrate (3)

Lithium 2-propanenitronate (83 mg, 0.87 mM) was flushed with argon for 30 minutes, then dissolved in DMSO (2.5 ml). Ethyl-1,1-dinitro-2-methylbutyrate (110 mg, 0.5 mM) in DMSO (2.5 ml) was added via a rubber septum and the solution irradiated with white light. An orange solution resulted almost immediately after mixing the reactants. The solution was stirred for 1 hour at room temperature, then ice-water added, causing some precipitation. The suspension was extracted with Et_2O in the usual way. Evaporation yielded pure 2,3-dimethyl-2,3-dinitrobutane (62 mg, 70% based on dinitro ester).

The aqueous solution was acidified with dilute HCl to pH 3 and extracted with

Et_2O. Evaporation and drying gave a blue oil (60 mg) which was purified by molecular distillation (75° (bath)/1 mm, lit.[80] b.p. 60° C/1 mm). Its spectroscopic properties confirmed its identity as ethyl-1-nitro-2-methylbutyrate (5).

n.m.r. ($CDCl_3$) : δ = 1.08 (d, 7, 6H), 1.40 (t, 7, 3H), 2.70 (m, 1H), 4.38 (q, 7, 2H), 4.86 (d, 9, 1H) (Double irradiation at δ 2.70 causes collapse of signals at δ 1.08 and 4.86 both to singlets)

i.r. (liq.film) : νcm^{-1} 2975 m s, 2940 m, 2880 w, 1750 s, 1565 s

M.S. : m/e 174 (≡ M^+ - H) Accurate mass 174.060, $C_7H_{12}NO_4$ ≡ 174.0766

129 (≡ M^+ - NO_2) Accurate mass 129.0922, $C_7H_{13}O_2$ ≡ 129.0915

43 (B)

With 1-Acetoxy-2,2-dinitrobutane (8)

1-Acetoxy-2,2-dinitrobutane (2.06 g, 10 mM) in DMSO (25 ml) was reacted with lithium 2-propanenitronate (1.65 g, 17.3 mM) in DMSO (50 ml) under argon in the usual way, with irradiation from a 20W fluorescent lamp. After 3 hours the reaction mixture was poured on to ice and extracted with Et_2O. Washing, drying and evaporation gave a brown oil (1.77 g) which contained 77% 2,3-dinitro-2-methyl-3-(acetoxymethyl)pentane (10) by n.m.r. (55% yield). The oil was crystallised from ethanol-pentane to give the pure compound (1.06, 43% isolated). Recrystallisation from ethanol-pentane gave white crystals.

m.p. : 61 - 62° C

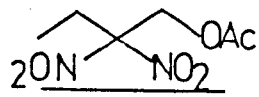
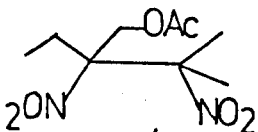
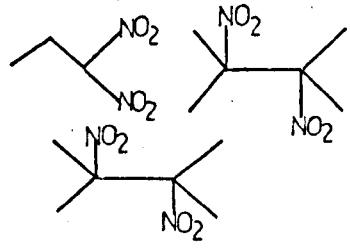
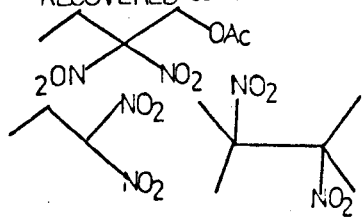
n.m.r. ($CDCl_3$) : δ = 0.94 (t, 7, 3H), 1.80 (S, 6H), 2.05 (S, 3H), 2.30 (m, 2H), 4.65 (q, 12, 18, 2H)

i.r. (CH_2Cl_2) : νcm^{-1} 2880 w, 1750 s, 1550 s

		% C	H	N
$C_9H_{16}N_2O_6$	requires :	43.54	6.50	11.29
	found :	43.42	6.26	11.20

The aqueous solution was acidified to pH 3 with dilute HCl and extracted as above to yield a blue oil (457 mg) which contained 1,1-dinitropropane (60% by n.m.r.) and 2,3-dimethyl-2,3-dinitrobutane.

Effects of Solvent and Reaction Conditions

Solvent	Mole Ratio $LiNO_2$ = 	Reaction Time Hours	 % †	Others
DMSO	1.1 : 1 ‡	2	44	
DMSO	1.1 : 1 ø	2	33	
DIOXAN	1.73 : 1	2	0	RECOVERED 83% 
EtOH	1.73 : 1	2	0	

† Calc. from n.m.r.

‡ Dinitro compound added to nitronate salt

ø Nitronate anion added to dinitro ester

With 2,2-Dinitro-1-pivaloyloxybutane (9)

Identical conditions and scale were used as described for the coupling between lithium 2-propanenitronate and acetoxy-2-2-dinitrobutane in DMSO. Reaction was allowed to proceed for 5 hours before pouring on to ice water and extracting with Et_2O. The crude product was a yellow oil (1.62 g, 77% pure by n.m.r., equivalent to 56% yield), which also contained 2,3-dimethyl-2,3-dinitrobutane. The latter crystallised preferentially from ethanol. Repeated recrystallisations (from pentane) were necessary to remove this impurity. The 2,3-dinitro-2-methyl-3-(pivaloyloxymethyl)pentane (11) was isolated as a white crystalline solid.

m.p. : 47° C

n.m.r. ($CDCl_3$) (100 MHz) : δ = 0.90 (t, 7, 3H), 1.20 (S, 9H), 1.76 (S, 3H), 1.82 (S, 3H), 2.36 (m, 2H), 4.64 (br S, 2H)

i.r. (CH_2Cl_2) : νcm^{-1} 2965 m s, 2910 m, 2875 m, 1738 s, 1548 vs

		% C	H	N
$C_{12}H_{22}N_2O_6$	requires:	49.64	7.64	9.65
	found:	49.50	7.69	9.51

Reactions Between Ring A Precursor (1) and 2,2-Dinitropropane

In Ethanol

The nitronate anion (1) was formed by addition of lithium ethoxide (3.62 ml of a 0.47 M solution in ethanol, 1.7 mM) to a solution of the parent nitro compound (453 mg, 1.5 mM) in ethanol (8 ml) under argon. 2,2-Dinitropropane (117 mg, 0.87 mM) in ethanol (5 ml) was added and the solution stirred and irradiated at room temperature. Periodically aliquots were removed, the solvent evaporated and the n.m.r. spectrum taken in $^{2}H_{4}$-methanol. After 6 days no coupled products were observed, unreacted 2,2-dinitropropane was present and some protonation of the anion had occurred, together with some decomposition.

In DMSO

The nitronate anion (1) was prepared identically to the above in ethanol and then the solvent removed in vacuo. The lithium salt was flushed with argon, dissolved in dry DMSO (10 ml) then reacted with 2,2-dinitropropane (117 mg, 0.87 mM) in the usual way. Removal of aliquots and work up at intervals indicated that several products were forming in very low yield. After 16 hours only starting material and unreacted 2,2-dinitropropane could be identified. No coupled products were present.

The course of the reaction was also followed on a small scale (0.1 mM) by ^{1}H n.m.r. in $^{2}H_{6}$-DMSO. A signal at δ 2.1 corresponding to the resonance due to acetone was formed during the reaction, although after 12 hours starting materials were still present. On work up, the acetone was not observed. No attempt was made to isolate it as its 2,4-dinitrophenylhydrazine derivative.

Synthetic Routes to Precursors (6) and (15)

Ethylene Ketal Nitro Acid Chloride (14)

Ethylene ketal nitro acid (13) (1.0 g, 3.7 mM) was suspended in dry benzene (15 ml) and freshly distilled thionyl chloride (8.7 g, 73 mM) under nitrogen, and stirred at 37° C. After 3 hours there was no unreacted acid present. Solvent and thionyl chloride in excess were removed in vacuo to give a white moisture-sensitive solid (1.08 g, 100%).

n.m.r. ($CDCl_3$) : δ = 1.30 (S, 3H), 1.49 (d, 7, 3H), 1.6 - 2.4 (m, 6H), 2.76 (q, 1H), 3.90 (S, 4H), 4.90 (q, 7, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 2960 w, 2890 w, 1801 s, 1550 s

Ethylene Ketal Trinitro Ester (6)

A satisfactory direct method for preparation of (6) was not developed.

The small quantity of (6) obtained was prepared by refluxing the acid chloride (14) (102 mg, 0.35 mM) with 2,2-dinitrobutanol (41 mg, 0.25 mM) in xylene (2 ml) containing magnesium powder (6 mg, 0.25 mM). After 4 hours the suspension was cooled and washed with dilute $NaHCO_3$, then water, and dried. Evaporation gave a dark brown oil (110 mg) which was a mixture of at least seven components. The ketal ester (6) was isolated from the mixture by p.l.c. (Sigel 0.75 mm ; CH_2Cl_2; $R_f \sim 0.3$) as a brown oil. The keto ester (15) had a very similar R_f and very careful separation was necessary. The oil was crystallised from ethanol-pentane (-78° C) to give an off-white solid (25 mg, 24%). This material was used for preliminary cyclisation studies but its preparation was not developed further.

n.m.r. ($CDCl_3$) : δ = 1.08 (t, 7, 3H), 1.27 (S, 3H), 1.47 (d, 7, 3H), 1.42 - 2.40 (m, 7H), 2.65 (q, 7, 2H), 3.90 (S, 4H), 4.85 (q, 7, 1H), 4.93 (S, 2H)

i.r. (CH_2Cl_2) : νcm^{-1} 3050 mw, 2970 m, 2890 w, 1754 s, 1573 s, 1548 s

Preparation of Trinitro Ester (15)

Nitro acid (16)

The ethylene ketal nitro acid (13) (3.0 g, 10.98 mM) was dissolved in a mixture of methanol (180 ml), water (50 ml) and concentrated HCl (25 ml, 35%) and the solution stirred at room temperature under N_2 for 19 hours. Methanol was removed in vacuo and the mixture was extracted with chloroform containing 10% ethylacetate. The extracts were washed well with water then dried. Evaporation yielded the nitro acid (16) (2.0 g, 77%) which was one component by t.l.c. Recrystallisation from ethanol gave colourless crystals.

m.p. : 167 - 168° C

n.m.r. (CD_3OD) : δ = 1.14 (S, 3H), 1.48 (d, 7, 3H), 1.95 - 2.8 (m, 7H), 5.06 (q, 7, 1H). $CO_2\underline{H}$ exchanged

i.r. (CH_2Cl_2) : νcm^{-1} 3020 mw, 2980 w, 1710 s, 1548 s

M.S. : m/e Accurate mass M^+ 229.0943; $C_{10}H_{15}NO_5$ requires M^+ 229.0950

		% C	H	N
$C_{10}H_{15}NO_5$	requires :	52.39	6.60	6.11
	found :	52.40	6.58	6.07

Nitro acid chloride (17)

The nitro acid (16) (1.9 g, 8.3 mM) was suspended in dry benzene (45 ml) and thionyl chloride (19.75 g, 166 mM), then stirred at 37° C under nitrogen. After 40 hours the reaction was still incomplete so the mixture was heated at reflux for 15 minutes, after which there was no acid present. The solvent and thionyl chloride were removed in vacuo to yield a brown crystalline moisture-sensitive solid (2.05 g, 100%). This material was not purified further and used directly.

n.m.r. ($CDCl_3$) : δ = 1.18 (S, 3H), 1.50 (d, 7, 3H), 2.10 - 2.80 (m, 6H), 3.14 (q, 1H), 4.98 (q, 7, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3050 mw, 2975 mw, 1810 sh, 1792 s, 1725 s, 1550 s

M.S. : m/e Accurate mass M^+ 247.0609; $C_{10}H_{14}NO_4{}^{35}Cl$ requires M^+ 247.0609

Trinitro ester (15)

Nitro acid chloride (17) (2.03 g, 8.2 mM) was mixed with 2,2-dinitrobutanol (2.02 g, 12.3 mM) and the stirred suspension was heated at 85 - 90° C under nitrogen. After $2\frac{1}{2}$ hours no acid chloride could be detected in the infrared spectrum of the reaction mixture which had blackened as soon as reaction began. The dark brown oil was dissolved in CH_2Cl_2 and filtered through silica gel (p.l.c. grade) to yield a brown oil (3.72 g). (Elution of the residue on the silica gel with methanol gave a brown gum (264 mg) which was predominantly the nitro acid (16)). The product was dissolved in CH_2Cl_2 and quickly washed with aqueous $NaHCO_3$ (353 mg, 4.2 M), then water to remove the excess dinitrobutanol. The solution was dried and then boiled with activated charcoal. Filtration and evaporation gave a viscous yellow oil which crystallised from ethanol. Repeated recrystallisation from ethanol was necessary to obtain the product as white crystals (1.50 g, 49%).

m.p. : 107 - 108° C

n.m.r. ($CDCl_3$) (100 MHz) : δ = 1.08 (t, 7, 3H), 1.17 (S, 3H), 1.48 (d, 7, 3H), 1.90 - 2.85 (m, 9H) (including 2.65 (q, 7, 2H)), 4.91 (q, 7, 1H), 4.99 (S, 2H)

i.r. (CH_2Cl_2) : νcm^{-1} 3050 mw, 2970 mw, 2890 w, 1755 s, 1715 s, 1572 s, 1548 s

u.v. (MeOH) : 226 nm

M.S. : m/e 329 ($M^+ - NO_2$) Accurate mass 329.1336; $C_{14}H_{21}N_2O_7$ requires 329.1349

	% C	H	N
$C_{14}H_{21}N_3O_9$ requires:	44.80	5.64	11.20
found:	44.96	5.79	11.06

Attempts at Cyclisation

Reaction of sodium methoxide with (6)

Ethylene ketal trinitro ester (6) (24 mg, 0.057 mM) was dissolved in CD_3OD (0.5 ml) under argon and $NaOCD_3$ (27.2 µl of a 2.1 M solution in CD_3OD, 0.057 mM) was added via a rubber septum and the reaction course observed by n.m.r. The solution instantaneously became yellow, indicating the formation of the 1-nitro-1-propanenitronate anion. Ester exchange was extremely fast and no cyclised products were observed. After 20 minutes the solution was evaporated to give a yellow solid which was extracted with CH_2Cl_2 to give a product, the n.m.r. spectrum of which confirmed substantial loss of the dinitrobutoxy group. The residue was acidified with dilute HCl and extracted to yield an oil (5 mg) which n.m.r. confirmed was 1,1-dinitropropane (n.m.r. ($CDCl_3$): δ = 1.13 (t, 7, 3H), 2.10 - 2.80 (m, 2H), 6.10 (t, 7, 1H)).

Reaction of (15) with sodium hydride in DMSO

The trinitro ester (15) (37.5 mg, 0.1 mM) was dissolved in dry $^2H_6$-DMSO (0.5 ml) and added to NaH (2.64 mg, 0.11 mM) in an n.m.r. tube. (The NaH was prepared by washing a 50% dispersion of sodium hydride in paraffin oil, with dry pentane . All manipulations were carried out in a dry N_2 glove box). Effervescence began immediately and the n.m.r. tube was irradiated with a 150W tungsten lamp. Removal of the nitromethine proton was slow and after 100 minutes, a second addition of NaH (2.64 mg, 0.11 mM) was made. Deprotonation was complete after 4 hours but the n.m.r. spectrum was, by this time, very badly resolved, suggesting some decomposition. After 6 hours the solution was diluted with ice-water and extracted with Et_2O. Drying and evaporation gave a brown oil (11 mg) which was at least four components. Separation was achieved by p.l.c. (Sigel 0.75 mm; CH_2Cl_2) but the starting ester was the only component which could be identified. Acidification of the aqueous solution with dilute HCl followed by extraction gave a brown oil (9 mg) which was at least four components by p.l.c. After separation,

none were identified. No 1,1-dinitropropane was observed.

Reaction of sodium amide with (15)

Sodium amide (9.17 mg, 85%, 0.24 mM) and the trinitro ester (15) were mixed in an n.m.r. tube under N_2 and dry dioxan added (0.5 ml). The n.m.r. spectrum was taken at intervals and when no reaction was observed after 14 hours at room temperature, the suspension was heated to 100° C, after which still no reaction occurred. The solvent was removed *in vacuo* and the residue dissolved in $^2H_6$-DMSO and warmed to 100° C causing extensive blackening and decomposition. The usual work up gave dark oils containing several products which were not identified.

Reaction of (15) with 'Proton Sponge'
(1,8-bis(dimethylamino)naphthalene

The trinitro ester (75 mg, 0.2 mM) and proton sponge (64.3 mg, 0.3 mM) were dissolved in $^2H_6$-DMSO (0.5 ml) under argon and there was no reaction at room temperature. The solution was heated to 95 - 100° C and after *ca.* 1 hour, it was apparent that epimerisation at the nitroethyl centre was occurring. Heating was discontinued and the solution was irradiated with a 150W tungsten lamp, the heat from which was used to maintain the temperature at *ca.* 60° C. After 3 days the n.m.r. spectrum was complex and badly resolved, suggesting decomposition. No cyclised products were evident.

Reaction of (15) with lithium 2,2,6,6-tetramethylpiperidide (LiTMP)

In HMPA

The trinitro ester (15) (75 mg, 0.2 mM) was dissolved in dry HMPA (0.5 ml) under argon in an n.m.r. tube so that the region ~5 p.p.m. could be examined. LiTMP was prepared by adding n-butyl lithium (87.6 µl of a 2.6 M solution in hexane, 0.22 mM) to 2,2,6,6-tetramethylpiperidine (31 mg, 0.22 mM) in HMPA (50 µl) under argon. The LiTMP solution was then introduced into the ester solution *via* a rubber septum. Deprotonation of the nitromethine carbon was instantaneous, so the solution was irradiated at room temperature. No further changes occurred in the n.m.r. spectrum after 30 minutes. After 1 hour the solution was added to ice-water and worked up as usual, yielding a yellow oil (10 mg). (Repeated extractions with Et_2O failed to increase the yield). The yellow oil was a mixture of compounds. The n.m.r. spectrum was not resolvable. The infrared spectrum

showed two NO_2 stretching frequencies (1573, 1550 cm^{-1}). Acidification of the aqueous solution with dilute HCl followed by extraction with Et_2O yield a brown oil (17 mg), the n.m.r. spectrum of which showed that it contained 1,1-dinitropropane (($CDCl_3$) δ = 6.10 (t, 7, 1H)).

In Dioxan

LiTMP (1.1 mM in dioxan, prepared by adding n-butyl lithium (647 µl of a 1.71 M solution in hexane, 1.1 mM) to 2,2,6,6-tetramethylpiperidine (155 mg, 1.1 mM) under argon) was added to a solution of the trinitro ester (15) (375 mg, 1 mM) in dry, peroxide free dioxan, giving a yellow solution. The solution was irradiated with a 20W fluorescent lamp and stirred at room temperature. After 3 hours the reaction was terminated by addition of ice-water and work up was as usual, yielding a brown oil (244 mg) which crystallised from ethanol giving only unreacted starting ester (15). Acidification of the aqueous solution and extraction with CH_2Cl_2 gave a second crop of starting material (56 mg) which also contained 1,1-dinitropropane. This latter product may have arisen from the basic aqueous work up conditions.

A separate n.m.r. experiment observing the region δ 0-3 indicated that LiTMP in dioxan did not deprotonate the ester (15) after 16 hours.

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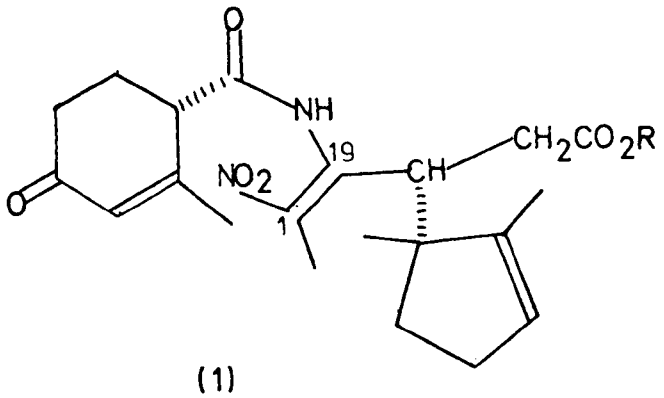
CHAPTER III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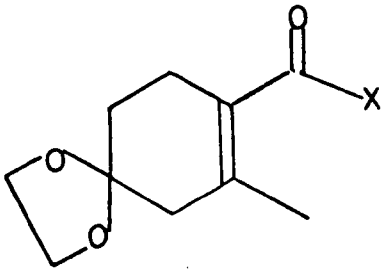
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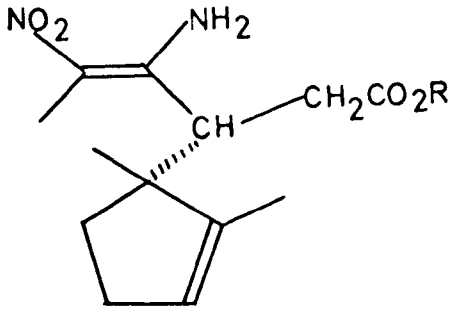
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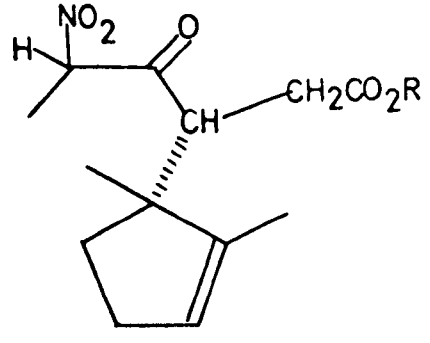
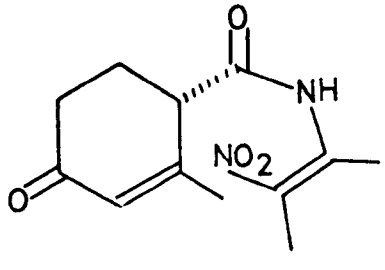
CH

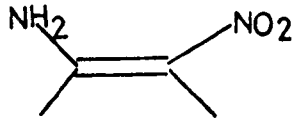
(3)


NO2
H
O
CH2CO2R
CH

(3a)


O
NH
NO2
O

(4)


NH2
NO2

(5)

Synthesis and Reactions of Amino- and Amido-Nitro-olefins

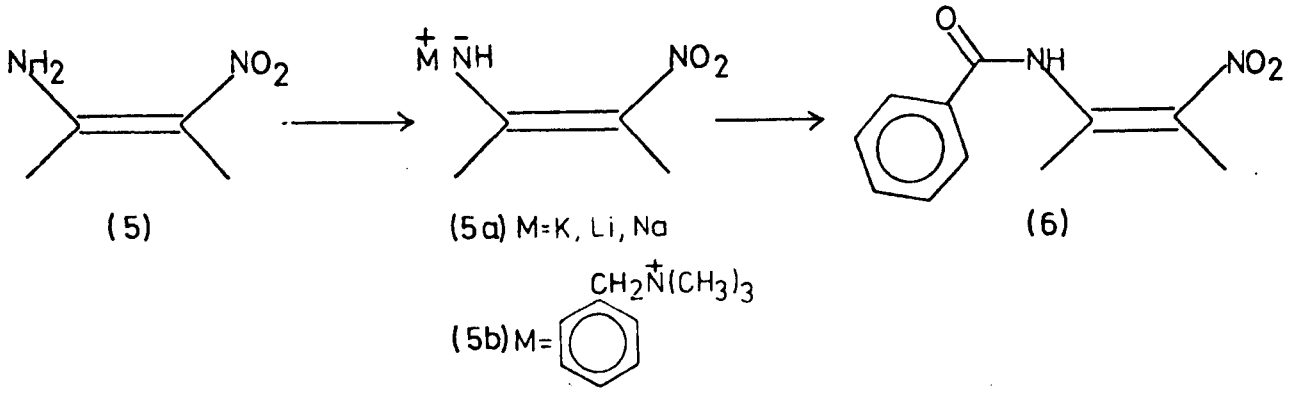
The limitations of radical-coupling reactions between nitronate anions and substituted nitro compounds described in chapters 1 and 2 ended this approach towards synthesis of the A/D component. The efforts invested in Hagemann's ester and its derivatives had been considerable, so it was desirable to use these intermediates as far as possible. Nitro-adducts were successfully prepared[17] by Michael addition of nitromethane to Hagemann's ester. Provided the bond which would ultimately become C1-19 in cobyric acid was already formed, it was possible that an intramolecular Michael addition could be used in the synthesis. The Michael addition was previously unsuccessful[15] for forming the C1-19 bond when using precursors (47) and (50). (Introduction page 6.)

The proposed intermediate incorporating the required functionalities for intramolecular addition was (1), and its synthesis could use ring A and ring D precursors already available. Ring D intermediate (3) was to be coupled with a reactive species such as (2). Initially, 2-amino-3-nitrobut-2-ene (5) was prepared as a model for (3) and some of its reactions were examined. (5) was readily synthesised by reacting ammonium acetate with 3-nitro-2-butanone, under acidic conditions; some nitroethane was identified as a byproduct. Reacting 3-nitro-2-butanone with 30% aqueous ammonia resulted in a mixture of nitroethane, acetamide and some (5), suggesting that under alkaline conditions, nitroethane is preferentially eliminated from the tetrahedral intermediate.

Reactions of 2-Amino-3-nitrobut-2-ene (5)

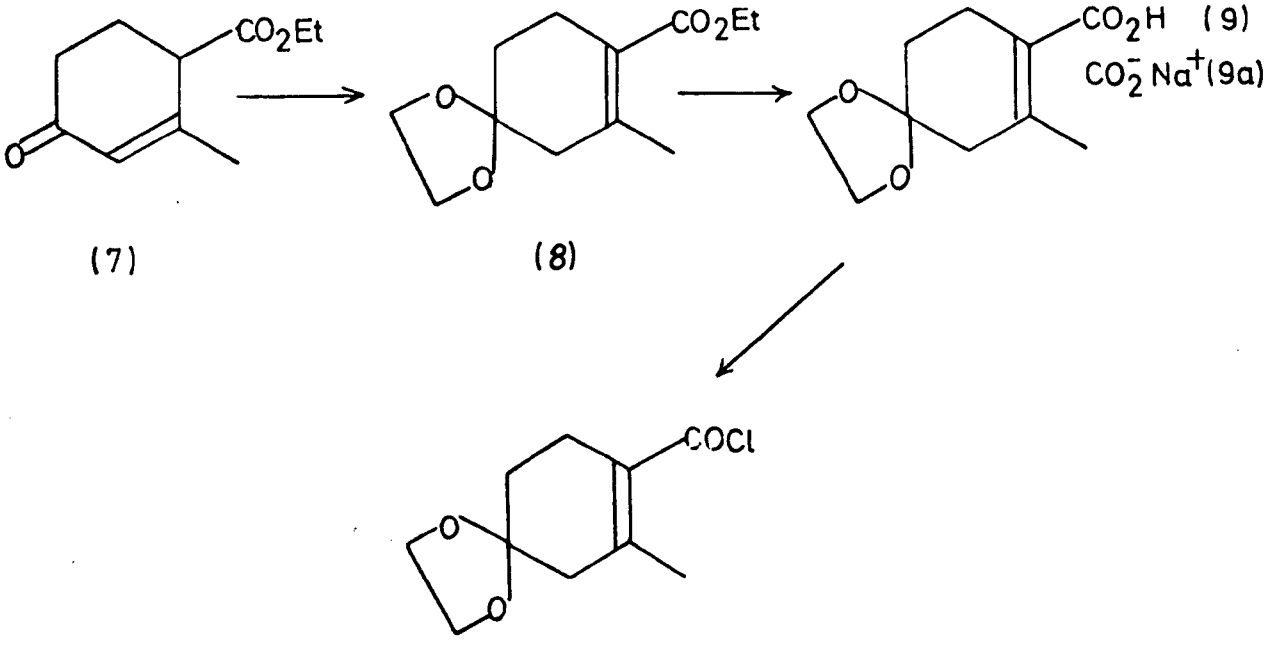
The configuration of the amino nitro-olefin (5) is cis (as shown) on the basis of infrared evidence.[81] (5) is stable in water but rapidly hydrolysed by dilute acid to 3-nitro-2-butanone. Not surprisingly, (5) was particularly difficult to acylate. For example, after heating with acetic anhydride and pyridine for several hours, no N-acetylated product was formed. Benzoyl chloride also failed to react satisfactorily. For a satisfactory synthesis of (1), it was necessary to find conditions whereby model amide derivatives of (5) could be prepared.

Sodium hydride is a convenient reagent for activating amides towards N-acylation. Treating (5) with sodium hydride in benzene gave a monosodium salt which was converted to the benzoyl derivative (6) on reacting with benzoyl

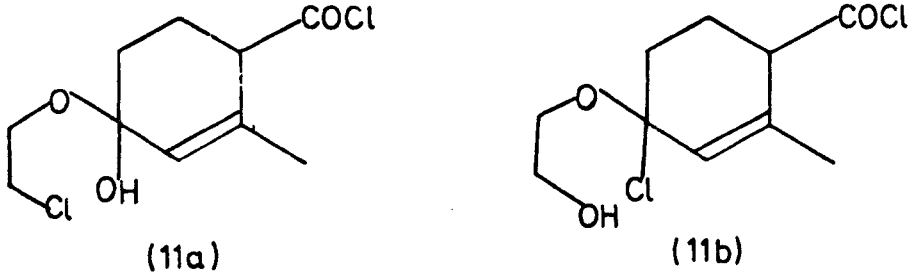


(5) (5a) M=K, Li, Na (6)

(5b) M= $CH_2\overset{+}{N}(CH_3)_3$



(7) (8) (10)



(11a) (11b)

SCHEME I

chloride. Some amino nitro-olefin (5) was recovered from the reaction and was presumed to have arisen from the product (6) protonating the unreacted salt of (5). Other bases were also satisfactory for forming the monosalt (5a). For example, potassium t-butoxide in benzene or lithium or sodium methoxide quantitatively gave (5a). Using these bases, (5a) could be converted to (6) in ca. 40% yield. Formation of the salt using n-butyl lithium followed by reaction with benzoyl chloride in dioxan, gave numerous products and only ca 20% yield of (6). Alkali metal salts (5a) were insoluble in benzene, so it was felt that the acylation might be enhanced using the benzyltrimethylammonium salt (5b) which was soluble in some organic solvents. In THF (5b) reacted slowly with benzoyl chloride and the product (6) was much more difficult to purify than in earlier successful experiments. An alternative route to amido nitro-olefins of the type (6) was by reaction of an amide with an α-nitro ketone. However, after refluxing benzamide and 3-nitro-2-butanone in benzene with a catalytic quantity of p-toluene sulphonic acid for long periods using a Dean-Stark water separator, only a very low yield of (6) resulted. Careful p.l.c. was necessary for its separation from the many byproducts. One significant by-product identified was N-acetylbenzamide, which probably arises through elimination of nitro-ethane rather than water from the tetrahedral intermediate.

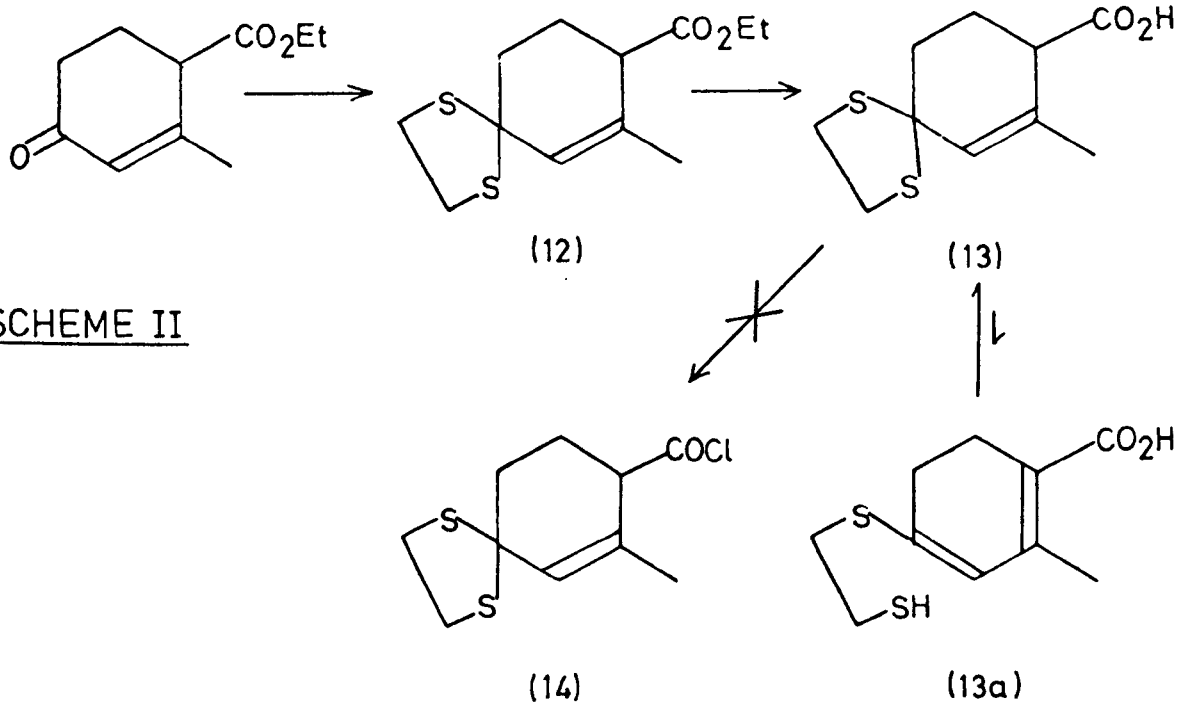
The benzamido nitro-olefin (6) was not hydrolysed readily, in contrast to the amino nitro-olefin (5). There was no change in the u.v. spectrum of (6) after standing in an acidic solution of n-propanol and water for 3 hours. Compound (6) was rapidly deprotonated at the amide nitrogen by methoxide ion in methanol and the deprotonated species showed a strong λ_{max} at 229.5 nm which is typical of nitronate anions.[51] Reprotonation of this anion regenerated compound (6). Hence, provided (4) could be synthesised, this suggested that the amide nitrogen would be sufficiently acidic to facilitate anion formation.

The key to acylation of compounds of type (5) appeared to be via the mono anion. Instead of optimising this reaction, it seemed appropriate to synthesise a reactive intermediate of type (2), so that amide formation with ring A precursors could be attempted, with the objective of preparing the model precursor (4).

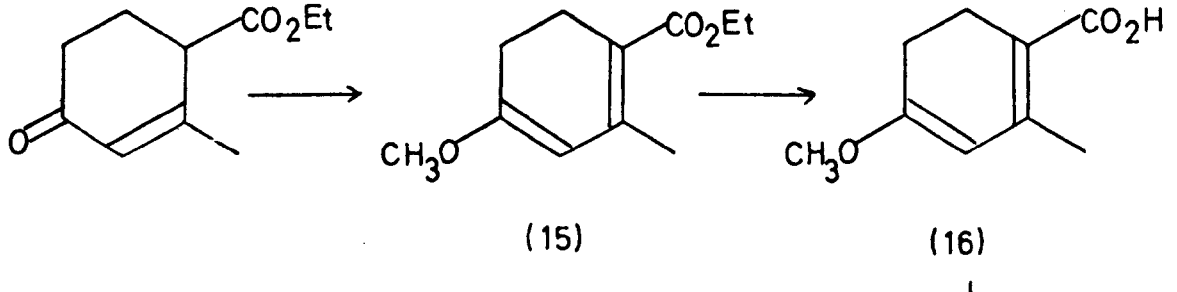
Acid Chlorides

Acid chlorides were previously used successfully in amide (chapter 1) and ester (chapter 2) syntheses, so Scheme I was the obvious course to examine. The

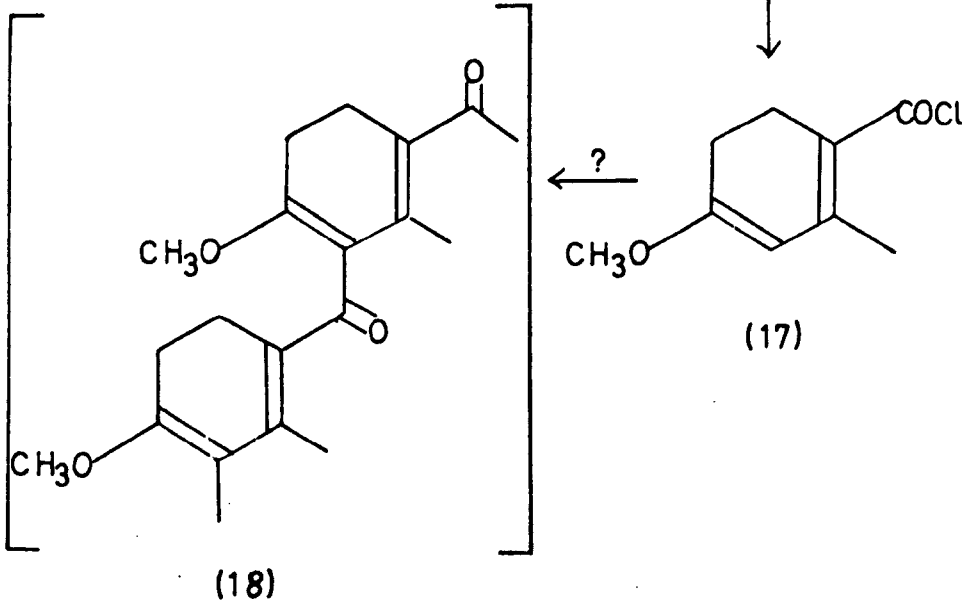
keto group of Hagemann's ester (7) required protection to prevent decarboxylation after hydrolysis of the ester. Ketalisation was effected smoothly, leading to largely one isomer (8) having the double bond in conjugation with the carboethoxy group. The ketal ester (8) was then converted to the corresponding carboxylic acid (9). Attempts to prepare the acid chloride (10) were not quite so successful. One of the difficulties lay in the lability of the ethylene ketal group towards acidic reagents. Freshly distilled thionyl chloride had been successful for preparing acid chlorides from carboxylic acids containing an ethylene ketal group, provided anhydrous conditions were employed (cf. chapters 1 and 2). However, with the carboxylic acid (9), thionyl chloride caused opening of the ethylene ketal. If ring opening had been a clean conversion to an intermediate of type (11), then this may have been of some use. Unfortunately this was not the case as the product was a complex mixture of compounds. Methods designed to eliminate hydrogen chloride formation, for example, by use of the sodium salt (9a) of the acid, were equally unsuccessful. The thionyl chloride method is known to fail with some carboxylic acids and all sulphonic acids, but dimethyl formamide catalyses[82] both reactions, either when used as a solvent or when used in catalytic quantities in an inert solvent. Using a variety of conditions with DMF, either the acid or the sodium salt were not converted to pure acid chloride, and n.m.r. evidence indicated that the ethylene ketal opened. The use of HMPA as a solvent for reactions with thionyl chloride has also been reported,[83] but this method was unsuccessful with acid (9). By contrast, dimethyl acrylic acid (21) was readily converted to its acid chloride (22) by thionyl chloride alone.[84] Other literature[85,86] methods also failed to give the pure acid chloride (10). Phosphorus pentachloride in chloroform again caused attack of the ethylene ketal and the mild non-acidic reagent, triphenylphosphine in carbon tetrachloride did not afford acid chloride. Further attempts at isolating pure acid chloride (10) were thus discontinued and a replacement for the labile ethylene ketal protecting group was sought. Ethylene thio ketals are usually much more resistant to acid than their oxygen analogues, so the ethylene thio ketal ester (12) was prepared. The preferred method of thio ketal group removal is normally by treatment with a mercuric salt.[87] Interestingly, although (13) was mainly one isomer, the double bond was not in conjugation with the ester carbonyl group.[88] The ester was converted to the corresponding acid (13) by alkaline



SCHEME II



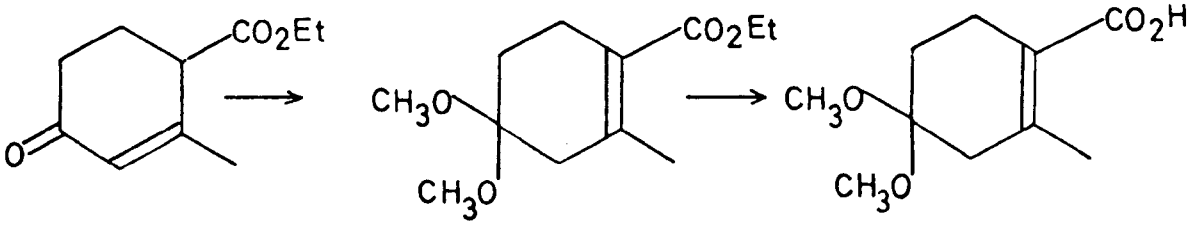
SCHEME III



hydrolysis. After recrystallisation of the acid, a close examination of the ^{1}H n.m.r. spectrum suggested that two isomers might be present as indicated by two signals for a vinylic proton at very similar chemical shift. It is possible that the acid (13) could self-catalyse ring opening of the thio ketal to give the isomer (13a). Its instability towards acidic reagents was certainly demonstrated on treating (13) with thionyl chloride. The mixture rapidly turned green, then dark brown, and on work up the n.m.r. spectrum of the product was completely unresolvable. A reaction monitored by n.m.r. in dilute solution in deuterochloroform showed that decomposition occurred very quickly. The signal due to the thio ketal methylene protons diminished within minutes, and after 45 minutes decomposition was complete. None of the products from this reaction could be identified. It is likely that if an equilibrium exists between (13) and (13a), then the free SH group will also react with thionyl chloride. Reacting the acid (13) with triphenylphosphine and carbon tetrachloride failed to give the acid chloride (14). After these setbacks it was not considered worthwhile to develop this scheme any further.

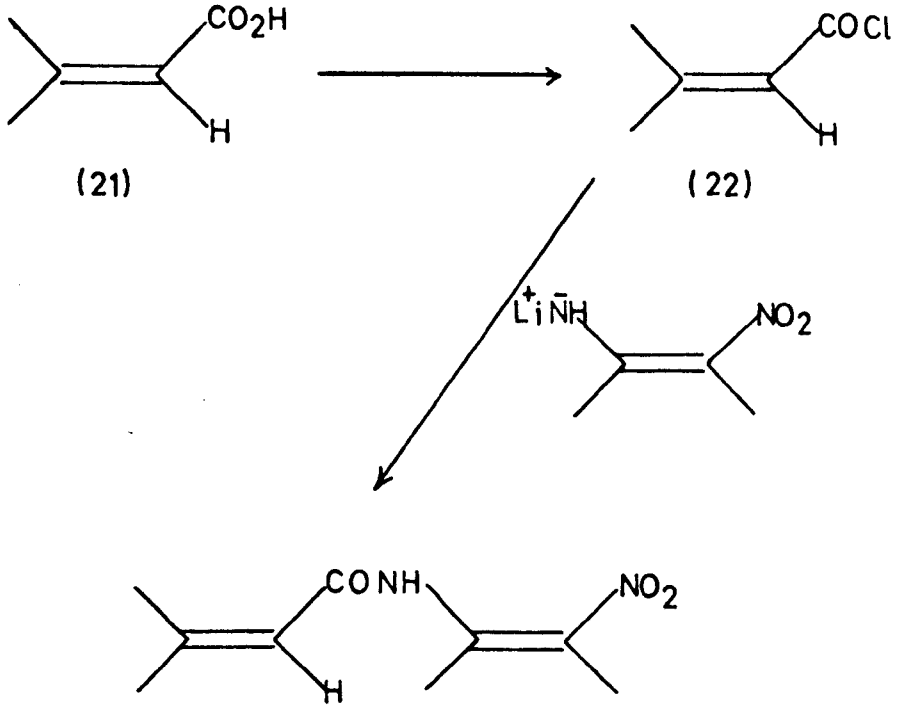
Another approach was examined simultaneously - that of Scheme III. Hagemann's ester was readily O-alkylated by reacting its enolate anion in DMSO with dimethylsulphate. Acid hydrolysis of the enolmethyl ether (15) only gave Hagemann's ester. Alkaline hydrolysis, however, converted the ester to the carboxylic acid (16). Thionyl chloride in benzene reacted vigorously with the acid (16) but it was evident that decomposition was occurring. When the reaction ceased no products could be identified but the main component appeared to be a polymeric gum. The carboxylic acid (16) reacted slowly with triphenylphosphine and carbon tetrachloride. N.m.r. observations showed a gradual diminution in the signals due to the acidic proton and the vinylic ring proton in (16). After 27 hours both had disappeared completely. The infrared spectrum of the product was not consistent with that of an acid chloride. A possibility is that as the acid chloride formed (17) it reacted with starting materials to give polymers of the type (18). It was not evident how to overcome this difficulty with this type of intermediate.

For completeness the dimethyl ketal, as a carbonyl protecting group, was examined. Hagemann's ester was converted to (19) by trimethylorthoformate



(19) (20)

SCHEME IV



(21) (22)

(23)

in methanol. Some of the enol ether (15) was also formed and this was not easily separable by chromatography. It was anticipated that distillation of (19) would result in conversion to (15), so the crude ester was directly hydrolysed to the acid (20). The hydrolysis was not as clean as others in this series had been, and after refluxing the ester for 40 hours in dioxan with aqueous sodium hydroxide, unreacted ester was still present. On work up some polymeric material was encountered, resulting in low yield of the dimethyl ketal acid (20), which was difficult to crystallise. The compounds in this series were again susceptible to acidic conditions and since (20) was not prepared easily in good yield, no attempt was made to prepare the corresponding acid chloride.

Before abandoning the acid chloride approach completely, the acid chloride (22), prepared from dimethylacrylic acid (21), was reacted with the lithium salt of aminonitrobutene (5) in benzene. The reaction was much slower than when using benzoyl chloride (complete in about 30 minutes) and was allowed to continue for 9 hours. After work up, t.l.c. was necessary to separate the products and the amide (23) was isolated in only 6% yield. This suggested that even if an acid chloride of the ring A precursor could be synthesised, then it also would probably be particularly unreactive.

Methods for Synthesis of Amides

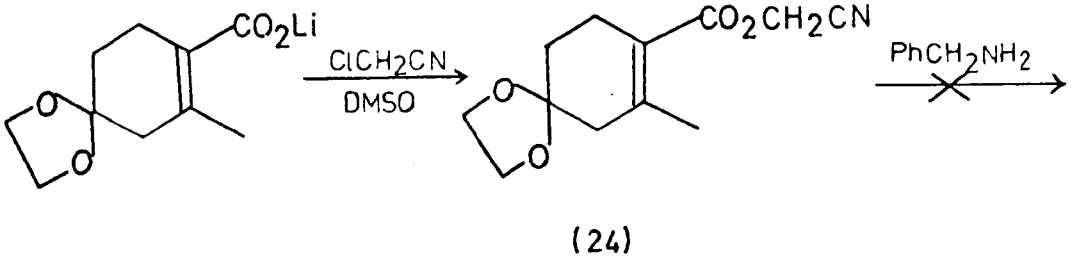
The difficulties encountered in the N-acylation of the aminonitro olefin (5) and in preparing a suitable acid chloride as precursor of the model compound (4), encouraged an examination of general methods for amide synthesis.

Activated esters

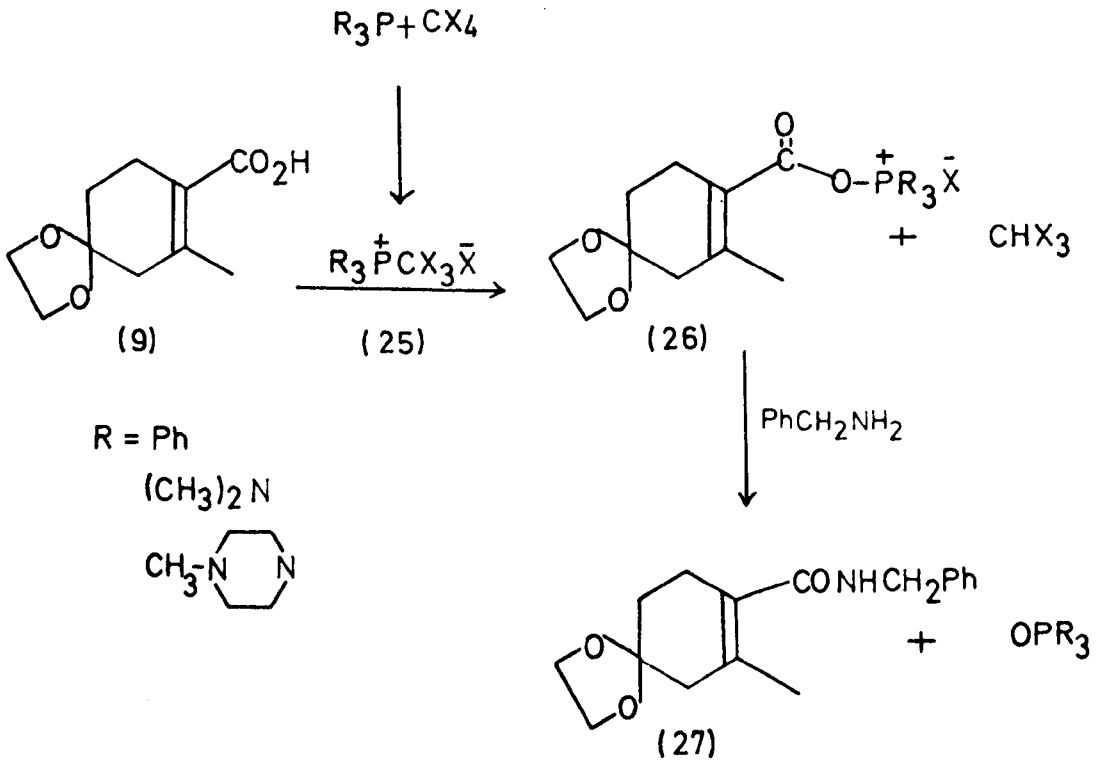
A large number of activated esters[89] have been proposed for use in peptide synthesis, although few have been employed in actual syntheses. The cyano ester (24) was prepared in good yield by reacting the lithium salt of (9) with chloroacetonitrile in DMSO. However, it was remarkably unreactive towards nucleophiles. For example, even after heating the cyano ester directly with benzylamine at 70° C for 48 hours, there was no reaction whatsoever. Conditions were not found whereby the ester could be induced to react. The lithium salt of aminonitrobutene was stirred with the cyano ester in DMSO but, again, there was no reaction.

Acyloxophosphonium Intermediates

Various methods of acylating amino components have been developed[90,91]

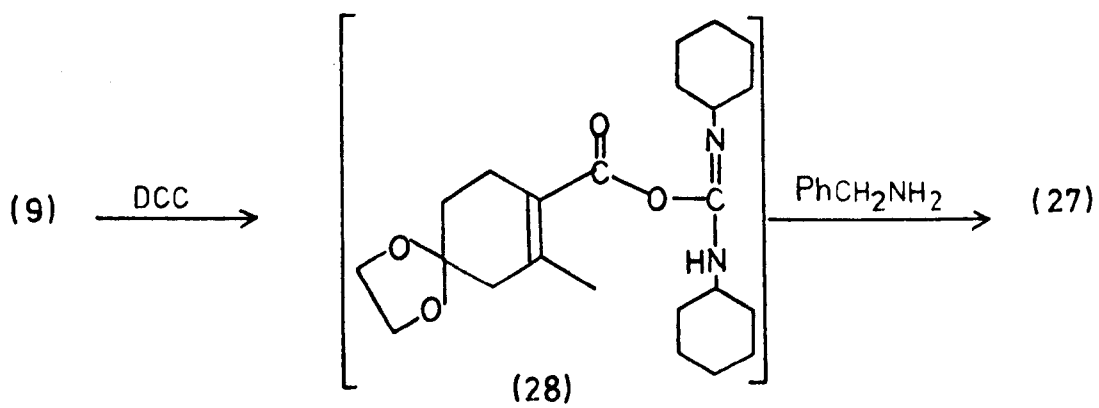


(24)

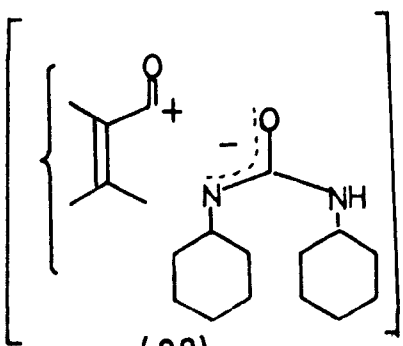


(9) (25) (26) (27)

SCHEME V



(28)



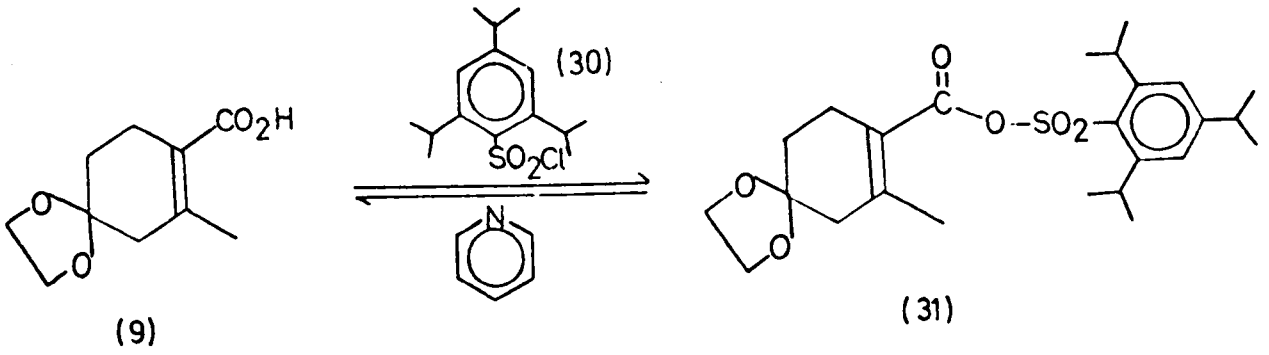
(29)

using acyloxophosphonium salts. Although this approach had failed to produce the ketal acid chloride (10), it was feasible that an acyloxophosphonium derivative (26) of the acid (9) might be prepared as in Scheme V. Provided this intermediate formed satisfactorily, then its carbonyl group should be activated enough to react with amines. Triphenylphosphine and carbon tetrachloride were converted to species (25), which reacted with the acid (9). Subsequent reaction with benzylamine effected conversion to the amide (27), but the yield was low and considerable difficulty was encountered in separating the product from the triphenylphosphine oxide also formed. However, if this method proved to be successful for acylating the aminonitro olefin (5), the latter problem could be avoided by the use of a different phosphine. For example, where R = (Me_2N) or N-methylpiperazino, the oxidated phosphoric amides are generally easily removed by dilute aqueous acid.

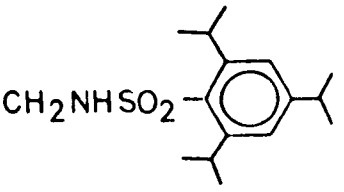
When benzylamine was replaced by the sodium salt of aminonitrobutene, conversion to the corresponding amide was unsuccessful. The only products identified from the reaction were unreacted amino compound (5) and, after acidification, the ketal acid (9). It is uncertain how the aminonitro olefin may have arisen. Either formation of the intermediate (26) was not quantitative, allowing acidic material to reprotonate the salt, or the anion was protonated *via* another source. The chloroform produced during formation of the acyloxophosphonium intermediate is a possibility. Conditions were not found by which (4) could be prepared using this method.

Dicyclohexylcarbodiimide (DCC) Derivatives[92]

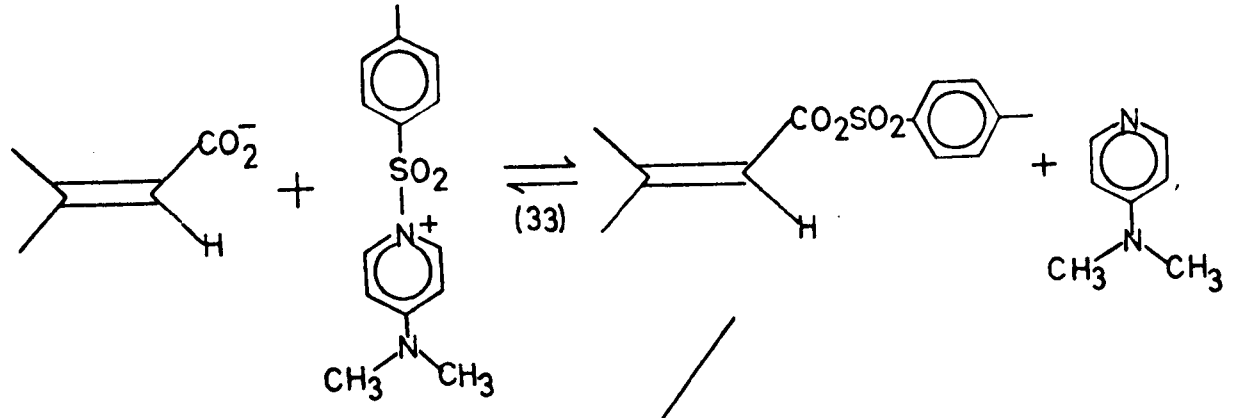
Mixing the ketal acid (9) with dicyclohexylcarbodiimide followed by addition of benzylamine was successful in forming the benzylamide (27). Although the spectroscopic properties of this compound were satisfactory, the material obtained was particularly difficult to crystallise even after chromatography. When the unsaturated acid (9) was mixed with DCC and addition of the amine component delayed, a white, very insoluble material precipitated. It was too insoluble to examine its spectroscopic properties fully, but from limited data it was concluded that it arose from *trans*-acylation in the intermediate (28). The nature of the intermediate in DCC reactions has yet to be completely defined,[93] although in solution the O-acyl-isourea as originally postulated[94] by Khorana appears to be the actual acylating agent. When dimethyl acrylic acid (21) was mixed with DCC, the same insoluble product resulted. In polar solvents, formation of an acylium cation from α,β-

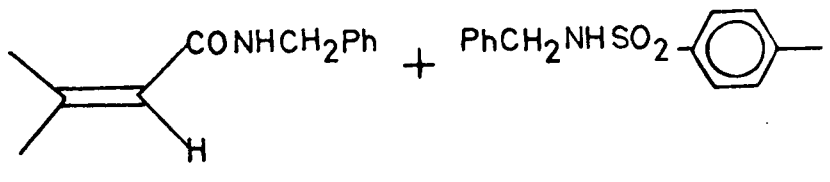


(9) (31)



(27) (32)





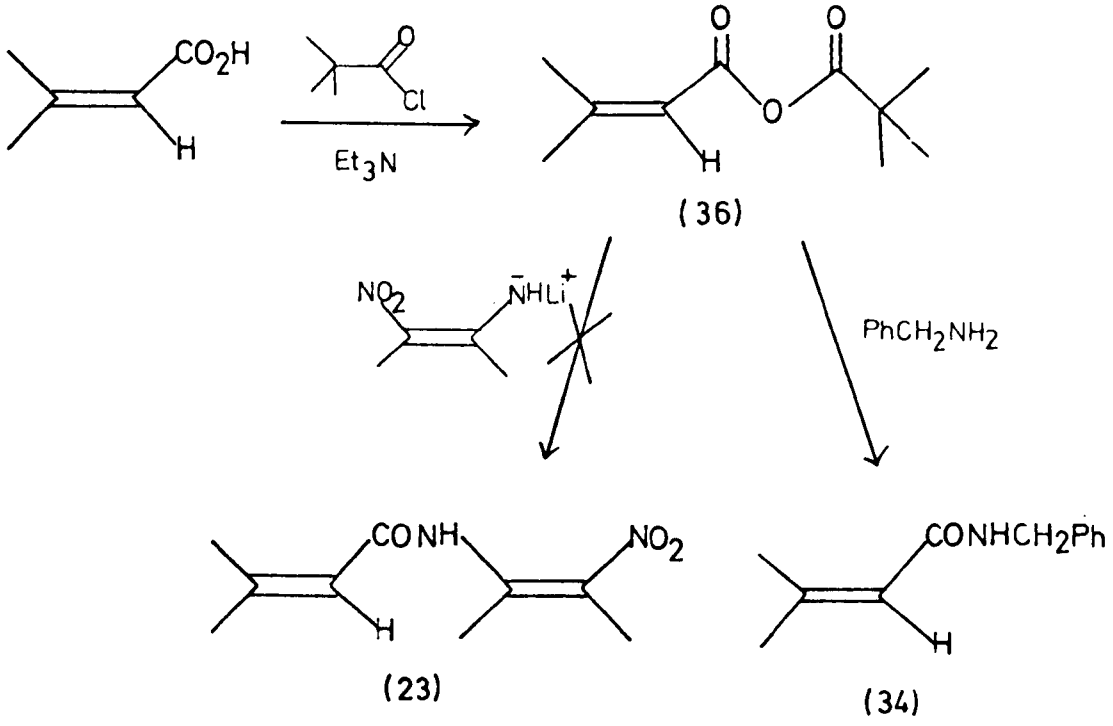
(34) (35)

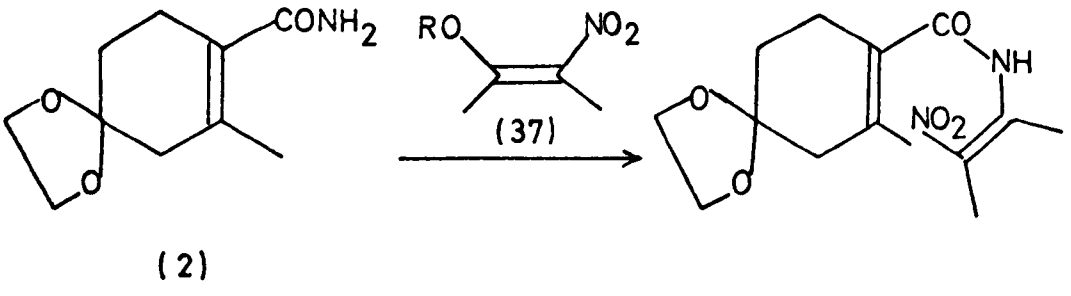
unsaturated acids may be promoted. This species may even exist as an ion pair, e.g. (29), which would greatly enhance the possibility of intramolecular acylation. Such reactions could, no doubt, be minimised by the use of low temperatures and apolar solvents such as carbon tetrachloride. Attempts were not made to develop the DCC reaction for acylation of the aminonitrobutene.

Aromatic Sulphonyl Derivatives

Khorana has successfully used arylsulphonyl derivatives in nucleotide synthesis[95] and essentially the method can be used for activating carboxylic acids towards attack by amines.

The ketal acid (9) reacted only slowly with 2,4,6-tri-isopropylsulphonyl chloride (TPS) in pyridine at room temperature to give the sulphonic anhydride (31), but after 20 hours the reaction reached an equilibrium. Warming to 100° C decomposed the sulphonic anhydride. If the equilibrium mixture containing the sulphonic anhydride was treated with benzylamine then the α,β-unsaturated amide (27) was produced together with the sulphonamide (32), which could be separated by chromatography. This result was sufficiently interesting to warrant a closer examination and a series of experiments was performed using various conditions, with dimethylacrylic acid (21) as a model α,β-unsaturated acid. I.r. spectroscopy was a convenient technique for following the course of these reactions, since the appearance of a new band at $\sim 1780\ cm^{-1}$ was indicative of sulphonic anhydride formation. Dimethylacrylic acid mixed with molar equivalents of N,N-dimethylamino pyridine and p-toluenesulphonyl chloride in dichloromethane, gave a sulphonic anhydride with a carbonyl absorption at $1775\ cm^{-1}$. The intensity of this absorption increased steadily to a maximum after about 30 minutes, and then no more sulphonic anhydride was formed, even though starting materials were present. Replacement of dimethylamino pyridine by pyridine resulted in a similar equilibrium. Addition of benzylamine to the equilibrium mixture (33) gave both the amide (34) and sulphonamide (35). The use of TPS in pyridine did not allow complete formation of a sulphonic anhydride even after 24 hours and the 2,6-isopropyl groups did not prevent formation of some sulphonamide when benzylamine was added. It was anticipated that the salt of an acid should react completely with a sulphonyl chloride rather than form an equilibrium mixture. The lithium salt of dimethylacrylic acid however, did not react to completion with TPS. The difficulties that this method generated did not appear to be


CO2H
H
Et3N
Cl
O
O
O
H
(36)
NO2
NHLi
PhCH2NH2
CONH
NO2
H
(23)
CONHCH2Ph
H
(34)


CONH2
O
O
RO
NO2
(37)
CO
NH
NO2
O
O

(2)

easily resolved, so no further attempts were made to develop it for activating the unsaturated acid (9).

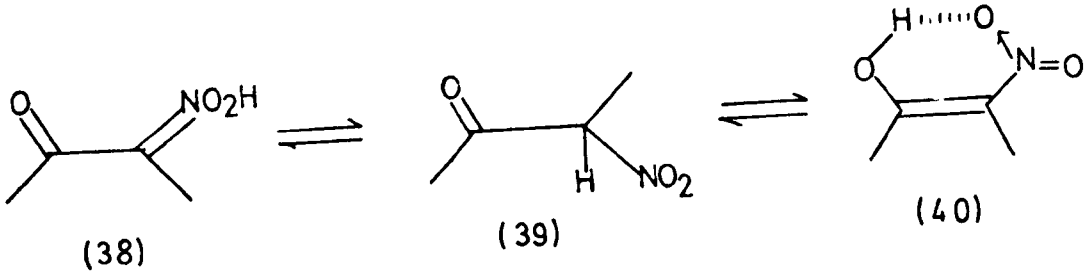
Mixed Carboxylic Acid Anhydride

The mixed anhydride method[96] is a convenient and well tried method for amide synthesis. To improve the selectivity of attack by the amino component, the activating acid chloride frequently chosen, is one with a hindered carbonyl group. Pivaloyl chloride is such an example and reacted rapidly with dimethylacrylic acid. and a molar equivalent of triethylamine, giving the mixed anhydride (36). This reacted with benzylamine to give only (34); there was no indication that pivaloyl benzamide had formed as a byproduct.

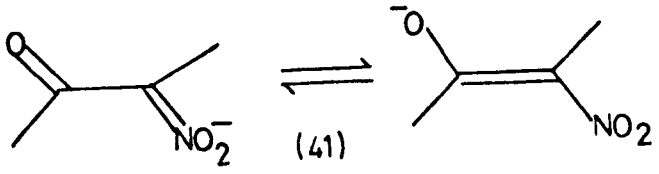
However, this method was not successful for acylating the lithium salt of aminonitrobutene. There was no reaction between the mixed anhydride (36) and the salt in dichloromethane. In acetonitrile, reaction was very slow and after 72 hours the reaction mixture was neutral and aminonitrobutene (5) was recovered. No attempt was made to apply the mixed anhydride method to acylations involving the unsaturated acid (9).

Because of the many chemical procedures[97] that have been used in peptide syntheses (for a review see reference 98) which might be applicable to the synthesis of intermediate (4), a systematic analysis of every method was not carried out. Nevertheless, the information gained from experiments performed, the weak nucleophilic nature of 2-amino-3-nitro-but-2-ene and the difficulties in acylating its salts, suggested that a synthesis of intermediate (4) was unlikely via this route. Also, even if the proposed ring D precursor (3) could be synthesised satisfactorily, then its linking to the ring A intermediate via an amide bond would probably be difficult, if not impossible. The other obvious route to the intermediate (1) is via the reaction of an amide ((2) where X = NH_2) with the α-nitroketone (3a). The failure to react benzamide with 3-nitro-2-butanone to give the amidonitro olefin (6) satisfactorily, suggested that this would not be a worthwhile alternative.

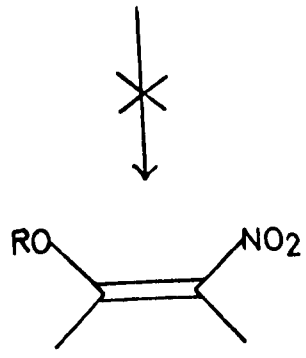
However, a modification of this approach is to react the intermediate (2) (where X = NH_2) with an electron deficient nitro-olefin such as (37). Attempts to realise this scheme were first directed to the synthesis of compounds of the type (37),



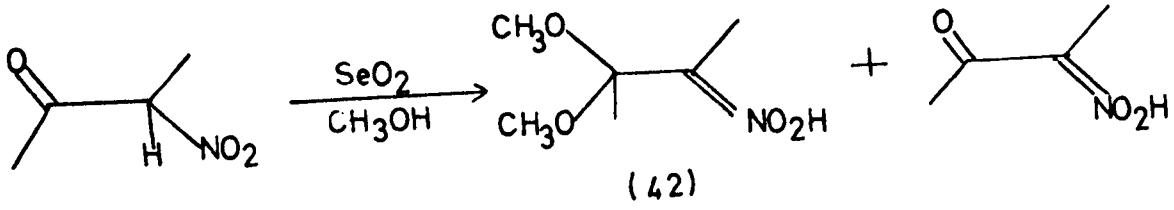
(38) (39) (40)



(41)



(37)

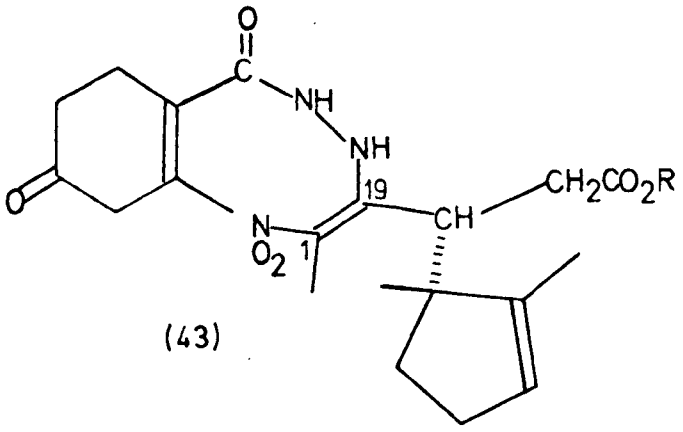


(42)

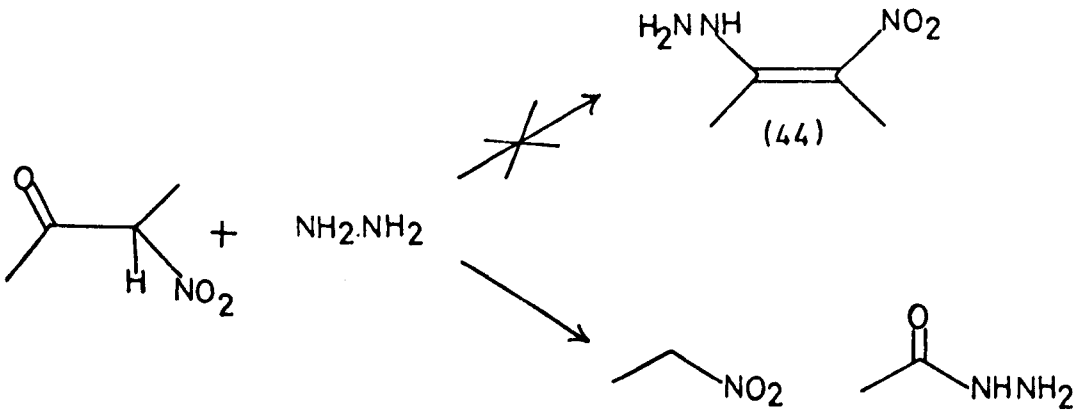
using 3-nitro-2-butanone as the chosen precursor. For all α-nitro ketones there exist three possible tautomeric forms : α-ketonitronic acid (38), keto (39) and enol (40), the interconversion being catalysed by acids and bases. In solution the three forms can exist in tautomeric equilibrium with the common ion (41). The composition of the equilibrium mixture is solvent dependent.[99] The keto form is favoured in polar protic solvents such as ethanol, whereas in aprotic solvents such as carbon tetrachloride or hexane the enol form is favoured, probably through intramolecular hydrogen bonding.[99] Its concentration is usually low. The concentration of α-ketonitronic acid present in these equilibria is also small. The anion (41) was easily prepared by addition of aqueous sodium hydroxide to 3-nitro-2-butanone. O-alkylation using dimethyl sulphate (methanol was added to give a homogeneous solution) was not successful for preparing (37) (R = Me). A mixture of products resulted, possibly via O-alkylation of the nitrogroup to give an unstable nitronic ester. Under similar conditions methyl iodide did not react. Formation of the anion (41) using sodium hydride in DMSO, followed by addition of methyl iodide, gave a product in which (37) could not be identified. This latter reaction was not examined extensively, although DMSO ought to have favoured O-methylation.

Triethylorthoformate reacts with ketones to give diethyl ketals.[100] This procedure has been modified[101] so that heating of the diethyl ketal leads to loss of a molecule of ethanol with the formation of the enol ether. Triethylorthoformate in ethanol did not react smoothly with 3-nitro-2-butanone using a variety of catalysts. In a reaction employing ferric chloride as catalyst and molar equivalents of triethylorthoformate and 3-nitro-2-butanone, at room temperature, unreacted nitro ketone was still present after $6\frac{1}{2}$ days. No nitrodiethyl ketal or nitroenol ether could be identified in the product. Heating a similar reaction mixture to reflux caused evolution of brown nitrous fumes, but after 2 hours reaction, nitro ketone was still present, although extensive decomposition had also occurred. The required product could not be identified in the mixture.

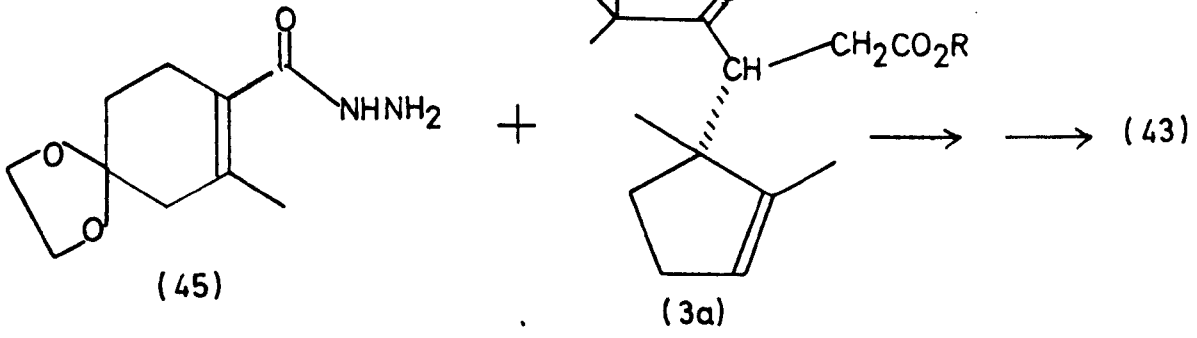
Selenium dioxide in methanol has been used effectively for forming dimethyl ketals of 3-keto steroids.[102] The selenium dioxide evidently functions as a dehydrating agent, and the selenious acid formed catalyses ketal formation. After refluxing 3-nitro-2-butanone for 24 hours with methanol and selenium dioxide, the



(43)



(44)



(45)

(3a)

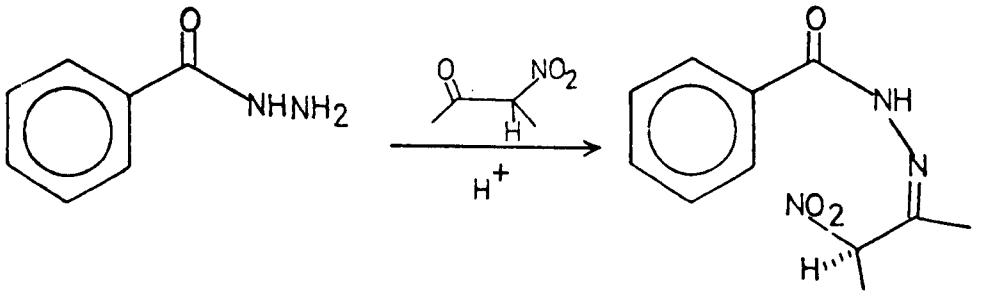
nitro ketone did not react to completion, and products in the reaction could not be completely characterised. In a similar reaction at room temperature, after 45 hours black metallic selenium precipitated, but by this time most of the ketone had reacted. Chromatographic separation afforded in very low yield a crystalline compound whose spectroscopic properties and combustion analysis were in accord with the nitronic acid structure (38). Complete separation of other fractions was not achieved and the contaminating selenium and/or selenious acid could not be removed, even after repeated chromatography. The n.m.r. spectrum of the crude product indicated that the dimethyl ketal nitronic acid (42) was the major component, but it was not possible to separate it pure. The unreactive nature of 3-nitro-2-butanone may be judged by its very slow reaction with 1,2-ethanediol. Refluxing the reagents in benzene for 116 hours with a catalytic amount of p-toluene sulphonic acid, failed to effect a complete conversion. It has been reported[103] that after 72 hours only a 36% yield of 3-nitro-2-butanone ethylene ketal can be obtained.

Because of the inability to easily synthesise model 2-alkoxy nitro-olefins, further attempts to incorporate this approach into a synthesis of the A/D component (1) were discontinued. The lack of specific reactivity that available precursors possessed manifested itself in the inability to use them for a successful synthesis of the model precursor (4). As a consequence, efforts directed towards the A/D component (1) were abandoned and some related systems were examined.

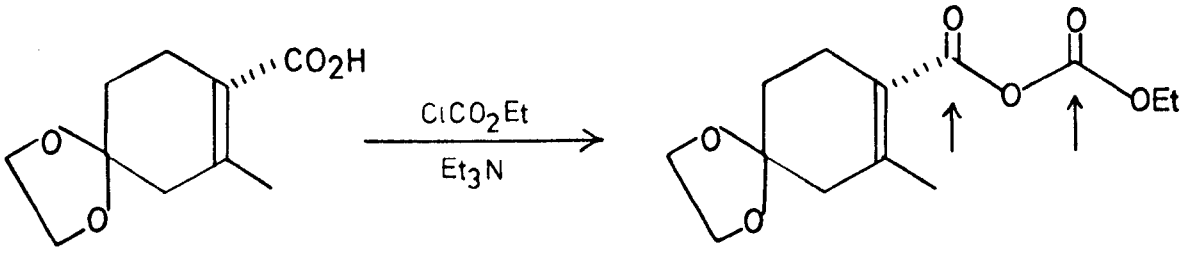
Synthesis and Reactions of Hydrazido Nitro-olefins

A related promising intermediate which could be synthesised from more reactive intermediates was compound (43). The hydrazine derivative (44) was chosen as a suitable model for a possible ring D precursor, but its synthesis in a similar manner to the aminonitrobutene (5) was impossible. The reaction of hydrazine with 3-nitro-2-butanone could be followed conveniently by n.m.r. spectroscopy. Whether the conditions were alkaline, neutral or acidic, nitroethane was always eliminated and no (44) was observed. It was concluded that the elimination of nitroethane from the tetrahedral intermediate was not dependent on pH, as N-acetylation occurred at all ranges of pH. This is in contrast to (5) which could be prepared in reasonable yield under acidic conditions by reaction of 3-nitro-2-butanone with ammonium acetate.

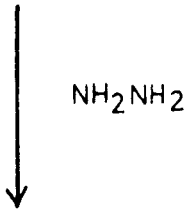
The alternative coupling procedure was to react the ring A hydrazide (45) with

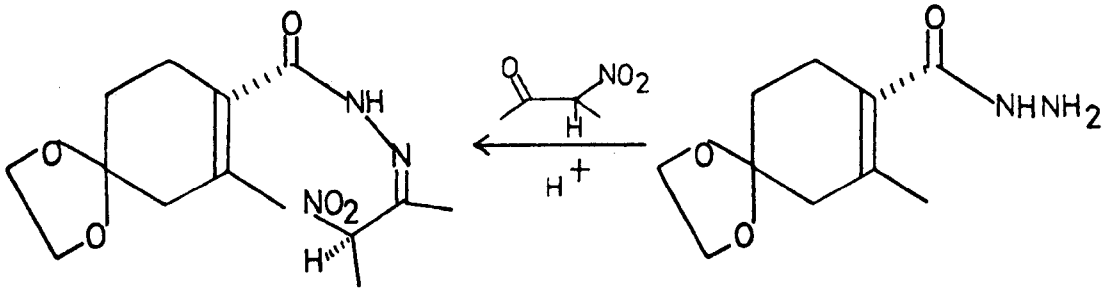


(46) (47)



(9) (48)

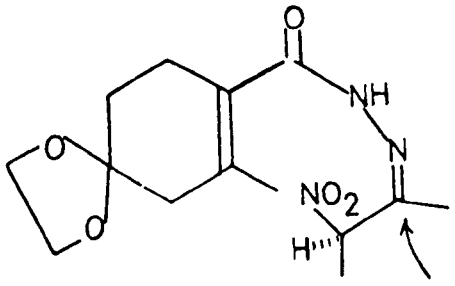




(49) (45)

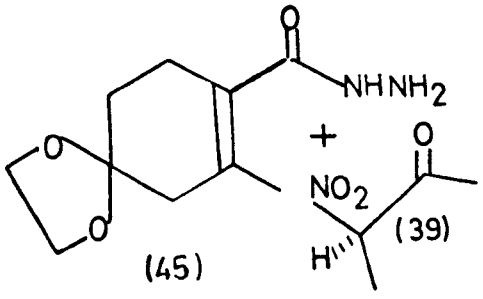
the ring D nitro ketone (3a). Hydrazides are normally readily available by treatment of esters[104] or acid chlorides[105] with hydrazine. Benzoyl chloride reacts with hydrazine to give benzhydrazide (46) which was used as a model to optimise conditions for reactions with 3-nitro-2-butanone. Reacting benzhydrazide (46) with 3-nitro-2-butanone under neutral conditions, led to the rapid formation of nitroethane. If the hydrazide was mixed with a molar equivalent of glacial acetic acid prior to reaction with the nitro ketone, then nitroethane was not eliminated. This result is consistent with observations made concerning the reactions of 3-nitro-2-butanone with ammonia (see page 64). Presumably protonation of the tetrahedral intermediate on oxygen must be a prerequisite for elimination of water, otherwise nitroethane is expelled. The anomalous behaviour of hydrazine has yet to be accounted for, but although hydrogen bonding between the amino and nitrogroups should be facilitated here, this ought to favour loss of water, rather than nitroethane. The product from the reaction of benzhydrazide with 3-nitro-2-butanone was obtained in good yield, but was not of the nitro-olefin structure expected, having the amidohydrazone structure (47). This was clearly evident from the n.m.r. spectrum which showed a methine quartet coupled to a doublet for a methyl group.

This encouraging result suggested that the ring A hydrazide (45) should react with 3-nitro-2-butanone and possibly the ring D nitro ketone (3a). Of course, the hydrazide (45) could not be synthesised *via* the acid chloride (10), as earlier attempts at preparing this compound were unsuccessful. Hydrazine reacts with esters to give hydrazides and because of the highly nucleophilic nature of hydrazine, little difficulty was anticipated in synthesising (45). However, the ketal ethyl ester (8) and the ketal cyanomethyl ester (24) were remarkably unreactive. Heating the ester (8) with hydrazine hydrate at 100° C for 48 hours failed to produce any hydrazide and unreacted ester was still present, together with many byproducts. After refluxing the cyanomethyl ester (24) with hydrazinehydrate in acetonitrile for 48 hours, no hydrazide could be identified in the mixture of products. The deactivation caused by the α,β-unsaturated system can be judged by these experiments, which offer further evidence accounting for the difficulties found in preparing amides in this series. Earlier attempts at preparing amides of the ketal acid (9) and dimethylacrylic acid (21) were useful here, in that activating methods which were unsuccessful previously could be discounted.

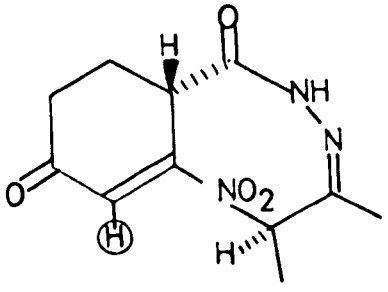


(49)

1Eq H^+ / H_2O

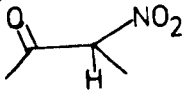


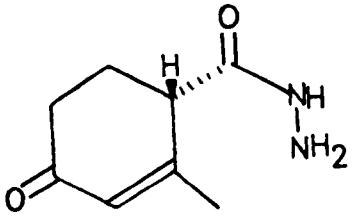
(45) (39)



(50)

?





(51)

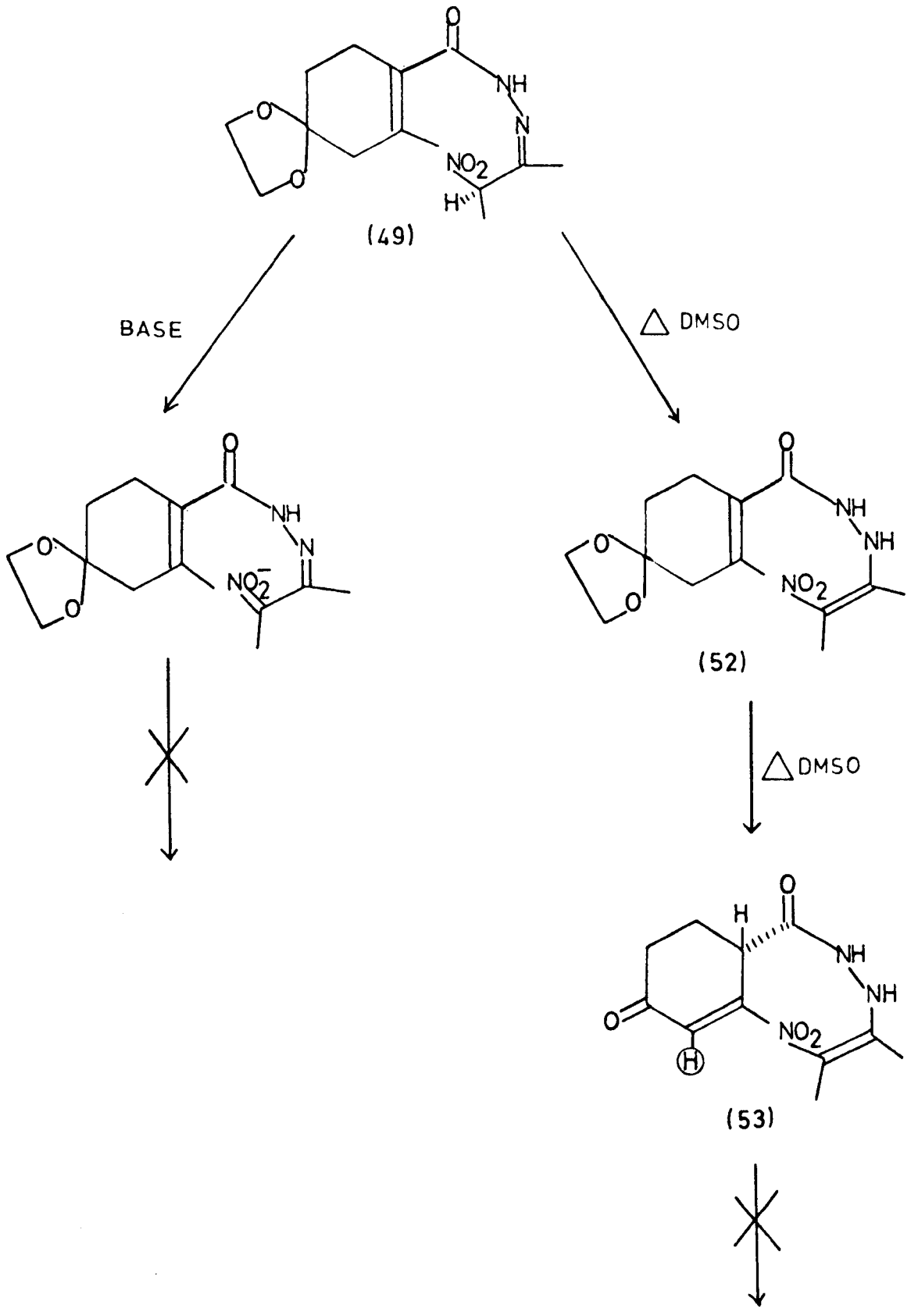
Pivaloyl chloride was successful in activating dimethylacrylic acid towards reaction with benzylamine. When used with the ketal acid (9), the mixed carboxylic acid anhydride formed satisfactorily, but reacting with hydrazine also caused attack at the pivaloyl carbonyl group.

The mixed carboxylic-carbonic acid anhydride method[106] for peptide synthesis has been well tried. However, it also suffers from the possibility of reaction at the wrong carbonyl carbon. The acid (9) reacted cleanly with ethyl chloroformate in acetonitrile, giving the mixed carboxylic-carbonic acid anhydride. Infrared spectroscopy was convenient for observing its formation and its subsequent reaction with hydrazine hydrate. Unfortunately hydrazine was not specific in its attack on the anhydride. Nonetheless, a procedure was developed by which the hydrazide (45) produced could be separated from the regenerated acid. (45) was extremely soluble in water so was isolated by a non-aqueous procedure in about 50% yield. The use of t-butylchloroformate for forming the mixed anhydride is a possible method of directing attack of the amine component to the required carbonyl, whereby improving the yield of (45).

The ketal hydrazide (45) reacted with 3-nitro-2-butanone in the presence of a molar equivalent of glacial acetic acid in benzene to give the model ring A/D precursor (49). Again, the model had the nitrohydrazone structure (49) rather than the nitro-olefin form.

Some Reactions of (49)

To carry out a normal Michael addition using this compound prior removal of the carbonyl protection was necessary. However, aqueous acidic hydrolysis, rather than removing the ethylene ketal group attacked the imine, regenerating the ketal hydrazide (45) and 3-nitro-2-butanone. The situation now reversed itself - this time when easy removal of the carbonyl protecting group was required, it turned out to be difficult due to other acid-labile functionalities within the molecule. The alternative carbonyl protecting groups that were examined earlier did not appear to offer distinct advantages, since much of their reactivity depended on other functionalities in the molecule (for example, the lability of the thio ketal group in the acid (13)). Systematic syntheses of derivatives of (49), each with a different keto protecting group would have been time-consuming and as there was no clear guarantee of success, this was not carried out. An alternative possibility would


(49)
BASE
DMSO
(52)
DMSO
(53)

have been to remove the ethylene ketal group in (45) to give the keto hydrazide (51), which could be reacted with 3-nitro-2-butanone. This approach suffered from the possibility of either an intramolecular cyclisation of (51) or even intermolecular addition occurring.

It was also possible that the ketal nitrohydrazone (49) could undergo cyclisation, perhaps via a Michael-type addition of its nitronate anion to the α,β-unsaturated hydrazide moiety. The nitronate anion of (49) formed readily in solution, as indicated by changes in the u.v. spectrum after adding base. The nitro compound had a broad maximum at 235 nm, whereas the nitronate anion exhibited three maxima at 233, 310 and 400 nm, but no changes occurred in this latter spectrum after several hours. Treatment of (49) with a catalytic quantity of sodium $^2H_4$-methoxide in $^2H_4$-methanol instantly gave a yellow solution. The signals in the n.m.r. spectrum of this solution, although at similar chemical shifts to the starting material, were considerably broadened. The solvent and TMS peaks were also broadened, suggesting the formation of a paramagnetic species. The signal due to the nitromethine proton had completely disappeared, although it may have exchanged with deuterium. No further changes occurred in this spectrum, although the yellow colour faded within 45 minutes. Neither warming the sample at 80° C, nor further standing at room temperature for 72 hours caused any change in the n.m.r. spectrum. There was no evidence to indicate that cyclisation was occurring.

Compound (49) underwent some interesting changes when warmed in DMSO. Monitoring its n.m.r. spectrum, (49) in $^2H_6$-DMSO was heated at 125° C, whereupon the quartet due to the nitromethine proton disappeared within 30 minutes. A new singlet at δ 3.45 also began to appear as the signal for the protons of the ethylene ketal ring diminished. On work up a low yield of a crystalline material was obtained, having spectroscopic properties consistent with the nitro-olefin structure (52). The strong NO_2 absorption at 1552 cm^{-1} present in the i.r. spectrum of (49) had disappeared, having moved perhaps to within a very broad absorption at 1675 cm^{-1} (shoulder at 1610 cm^{-1}). The movement of N=O absorption to higher wavenumbers is consistent with the formation of the nitro-olefin isomer (52). NH stretch was present at 3548 cm^{-1}. The mass spectrum showed no molecular ion, in contrast to (49). However, the fragmentation pattern was suggestive of having arisen from a molecular ion of (52), the highest ion being m/e 295 ($M^+ - CH_4$), and m/e 237 corresponded to (295 - CNO_2). The n.m.r. spectrum was not well resolved, but

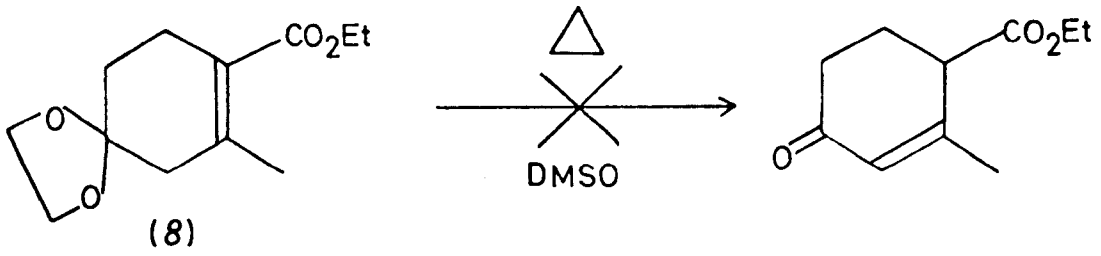
was in general agreement with the nitro-olefin assignment (52).

Some further experiments probed the scope of this interconversion. Standing (49) with a catalytic quantity of trifluoroacetic acid in $^2H_6$ - DMSO at 37°C, caused very slow isomerisation to the nitro-olefin form, but was only partially complete after 24 hrs. Also the singlet at δ 3.45, observed previously, was present in the n.m.r. spectrum. Heating this sample to 190°C for 10 minutes caused rapid blackening and complete loss of the signal at δ 3.96 due to the ethylene ketal protons, to be replaced in entirety by the singlet at δ 3.45. The chemical shift of this latter signal is consistent with that for the methylene protons of ethanediol, but it is uncertain how this may have arisen. Deketalisation to the α, β-unsaturated cyclohexanone system seemed fairly certain as a, narrow multiplet at δ 5.95, indicative of the vinylic proton, was present in the spectrum. The characteristic signals for the methyl groups in 3-nitro-2-butanone were also identified, suggesting that trifluoroacetate ion had attacked the hydrazone.

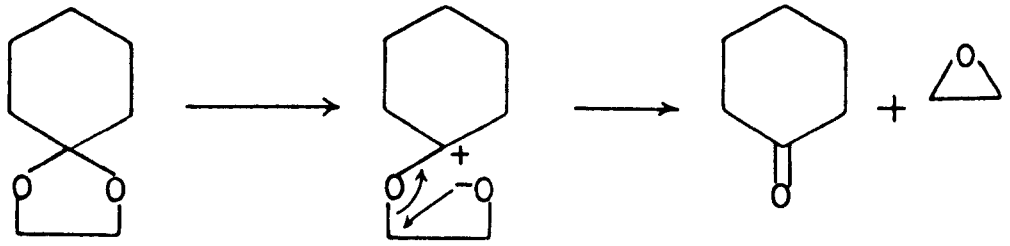
Heating (49) in $^2H_6$ - DMSO alone, at 190°, also effected deketalisation and the conversion was complete after 1 hr, although after this time the n.m.r. spectrum was badly resolved, but no 3-nitro-2-butanone was formed. The product from this reaction was a brown oil containing several components. Its infrared spectrum showed very broad absorptions in the carbonyl region; there was no NO_2 absorption at ~1550 cm^{-1} as this band may have moved to within the broad absorption at higher wavenumbers. The evidence suggested that deketalisation and isomerisation to the nitro-olefin form (53) had occurred, with some decomposition also. This decomposition precluded attempts to develop this method into a preparative route to (53). It was possible that (53) might cyclise via a Michael-type addition of the nitro-olefin to the α, β-unsaturated enone system. This had not taken place, as the α-proton in the enone was clearly observed in the n.m.r. spectrum of the product.

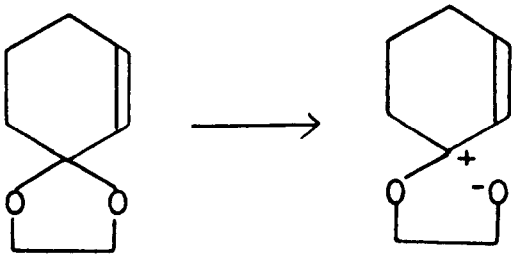
Reactions of Ethylene Ketals with DMSO

Heating ethylene ketals with DMSO was not a general reaction for the removal of the carbonyl-protection. The ethylene ketal unsaturated ester (8) showed no evidence of deketalisation after heating in $^2H_6$ - DMSO at 190°C for



(8)





SCHEME VI

2 hrs, monitoring the reaction by n.m.r. spectroscopy. It was thought that in polar solvents and at high temperatures, the ethylene ketal might open to give an ion pair, which might rearrange as shown in Scheme VI. Unsaturated systems would be expected to stabilise this ion; it is interesting that the unsaturated ester (8) was completely stable under these conditions. Cyclohexanone ethylene ketal reacted only partially after 2 hrs in $^{2}H_{6}$ - DMSO at 190°C; again the signal at δ 3.45 was seen in the n.m.r. spectrum. The mechanism outlined in Scheme VI requires the formation of ethylene oxide, but this was not observed. A similar experiment incorporating a catalytic amount of lithium perchlorate gave very similar results.

Conclusion

The synthetic difficulties involved in a route to the keto nitrohydrazone (50), a form more conducive to base-catalysed cyclisation, were numerous. Neither compound (50) or its nitro-olefin isomer (53) could be obtained in a pure form, in order to rigorously study conditions for a cyclisation.

Consequently there was no evidence to encourage attempts at the synthesis of the analogous precursor (43), containing both ring A and ring D intermediates. The results from these preliminary investigations suggested that this approach had only a small likelihood of success. Unfavourable thermodynamics could vitiate the proposed Michael addition, and, although this was realised at the outset, the problems that arose during the synthesis of precursors, of a suitable form for cyclisation, were not envisaged. These two factors disuaded further efforts in this area to realise the proposed scheme.

EXPERIMENTAL III

Preparation of 3-nitro-2-butanol

A general procedure for the preparation of nitroalcohols was used[103] which was a modification of Staub's method[107], involving base-catalysed addition of nitroethane to acetaldehyde.

b.p. 100-108°C/15 mm : (lit.[103] 80-87°/8 mm)

n.m.r. ($CDCl_3$) : δ = 1.25 (dd, 6, 3H), 1.5 (dd, 6, 3H)
3.04 (br, S, 1H), 3.90-4.70 (m, 2H)

i.r. (film) : ν cm^{-1} 3400 s, 2980 ms, 1548 s

Preparation of 3-nitro-2-butanone

A modified[103] procedure of that used by Levy and Scaife[108], involving oxidation of the nitro alcohol with sodium dichromate and conc. H_2SO_4, was used.

b.p. : 77-79°/15 mm (lit.[103] 71-75°/9 mm)

n.m.r. ($CDCl_3$) : δ = 1.73 (d, 7, 3H), 2.30 (S, 3H), 5.32 (q, 7, 1H)

i.r. (film) : ν cm^{-1} 2900 m, 1724 s, 1548 s

n_D^{19} : 1.433 (lit.[103] n_D^{20} 1.4349)

Preparation of cis 3-amino-2-nitro-but-2-ene (5)

3-nitro-2-butanone (5.05 g, 43 mM) and ammonium acetate (7.70 g, 100 mM) were dissolved in glacial HOAc (5 ml) and heated at 95°C for 30 minutes. The dark brown mixture was cooled and solid $NaHCO_3$ added to neutralise. The suspension was filtered and washed with CH_2Cl_2 and concentrating the filtrate caused crystallisation. The crystals (2.25 g, 45%) were filtered off, and the filtrate evaporated yielding a brown gum which was washed quickly with water, then extracted with CH_2Cl_2 to give a further 335 mg of crude product. The first crop of crystals were recrystallised from 3 : 2 EtOH/MeOH to yield pale yellow crystals (1.87 g, 37%). The residues from the crystallisation had an i.r. spectrum identical to the recrystallised material.

m.p. : 158-159°(lit.[81] 159-160°C)

n.m.r. (CD_3OD) : δ = 2.08 (S, 3H), 2.17 (S, 3H)

($CDCl_3$) : δ = 2.11 (S, 3H), 2.14 (S, 3H)

i.r. (CH_2Cl_2) : ν cm^{-1} 3420 s, 3220 w, 1610 s, 1545 w

u.v. (MeOH) : 355 nm (ε, 17,300) 235 nm (ε, 2,800)

		%C	H	N
$C_4H_8NO_2$	requires	41.37	6.94	24.13
	found	41.40	7.00	24.20

Reaction of 3-nitro-2-butanone with NH_4OH

NH_4OH (0.43 ml of 30% soln. of NH_3 in water, 3 mM) was added to 3-nitro-2-butanone (234 mg, 2 mM) in an n.m.r. tube. There was a vigorous reaction and the mixture was cooled in water. $CDCl_3$ (0.6 ml) was added and the mixture well shaken, then centrifuged. The n.m.r. spectrum was taken directly on the lower layer. Peaks corresponding to 3-amino-2-nitrobut-2-ene (δ 2.11 (S), 2.14 (S)), acetamide (δ 1.97 (S)) and nitroethane (δ 1.55 (t, 7), 4.40 (q, 7)) were present, and were in the ratio 20 : 15 : 65 respectively. The low figure for acetamide is presumably due to some remaining in the aqueous phase.

Acylation of 3-amino-2-nitro-but-2-ene (5)

With Acetic Anhydride

A mixture of (5) (174 mg, 1.5 mM), Ac_2O (1.37 g, 7.5 mM) and pyridine (0.1 ml) was heated at 95-100°C for 2 hrs. Excess Ac_2O was removed in vacuo; the oily residue dissolved in CH_2Cl_2 and the solution washed with dil $NaHCO_3$, water and dried. Evaporation gave a brown oil (70 mg) which was a mixture of products and could not be identified. There was no N-acetylated material present.

With Benzoylchloride

(5) (58 mg, 0.5 mM), PhCOCl (70 mg, 58.4 µl, 0.5 mM) and pyridine (39.5 mg, 40 µl, 0.5 mM) were dissolved in $CDCl_3$ (0.5 ml) and the solution maintained at 37°C. The n.m.r. spectrum of the mixture was recorded periodically, and after 23 hrs only weak signals for the N-benzoylated material could be observed. The solution was added to water, extracted with Et_2O, and extracts were washed well with water. Drying and evaporation gave a brown solid (78 mg). Recrystallisation (EtOH) gave a crystalline material whose identity was confirmed as benzoic acid, by comparison with authentic material (i.r. (CH_2Cl_2): ν cm^{-1} 2800 m, 2600 m, 2580 m, 1710 sh 1685 s, 1590 m, 1575 m). The residue showed peaks in the i.r. spectrum consistent with benzoic anhydride (i.r. (CH_2Cl_2): ν cm^{-1} 1785 s, 1720 s, 1680 m, 1598 m).

The N-benzoylated product (6) was not isolated.

Preparation of salts of (5)

Metal salts (5a)

Na Several methods were used:

(i) NaH (1 molar equivalent of a 50% dispersion in paraffin oil previously washed with dry pentane under N_2) was added to (5) in dry benzene forming a white suspension and allowed to react until effervescence ceased.

(ii) 1 molar equivalent of NaOEt in EtOH (or NaOMe in MeOH) was added to a solution of (5) in EtOH (or MeOH), and the solvent then removed in vacuo to yield a white solid. (When $NaOCD_3$ in CD_3OD was used, the protons on the methyl group α to the amino nitrogen, rapidly exchanged with deuterium, giving the trideuterated salt.)

Li The lithium salt was prepared by

(i) adding a molar equivalent of LiOEt to a solution of (5) in EtOH and the solvent removed in vacuo, or

(ii) adding n-butyl lithium (1 molar equivalent of a 2.1 M soln. in hexane) to (5) in dry dioxan.

K A suspension of molar equivalents of (5) and $KOBu^t$ were refluxed in dry benzene for 2 hrs then cooled.

Benzyltrimethylammonium methoxide salt (5b)

A molar equivalent of benzyltrimethylammonium methoxide (2.1 M soln. in MeOH) was added to a solution of (5) in MeOH. The solvent was removed in vacuo after stirring for 15 mins, yielding a hygroscopic off white solid, which was insoluble in CCl_4, soluble in THF, and, soluble in $CHCl_3$ but giving a deep red solution.

Preparation of N-(3-nitro-but-2-ene)-benzamide (6)

A molar equivalent of benzoylchloride was added to a suspension of a monosalt of (5) in an inert solvent. Solvents and temperature of addition were varied; benzene and room temperature were a satisfactory combination. Reaction was usually complete within ca. 1 hr at room temperature and further refluxing for 4 hrs did not improve the yield. Percentage yields of (6), according to reaction conditions, are given in Table I for the various salts. Reactions were worked up by evaporation, then adding CH_2Cl_2 and the suspensions

were filtered through silica gel (p.l.c. grade, ca. 1 g/1 mM) eluting with CH_2Cl_2, followed by a further evaporation. The crude products were recrystallised from EtOH to yield pure (6).

m.p.	:	108-109°C
n.m.r. (100 MHz) ($CDCl_3$)	:	δ = 2.29 (5, 3H), 2.71 (S, 3H), 7.55 (m, 3H) 7.97 (m, 2H), 10.6 (brS, 1H)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3600 vw, 1690 s, 1603 s,
u.v. (i) (MeOH)	:	246 nm (ε, 10,200), 358 nm (ε, 9,400)
(ii) (MeOH/NaOMe)	:	229.5 nm (ε ~16,300), 274 (7,500)
		Addition of 1 N HCl to (ii) gave spectrum (i)
(iii) ((1 : 1 n-propanol/ water)/HCl)	:	249 nm, 365 nm
		No change after 3 hours

		%C	H	N
$C_{11}H_{12}N_2O_3$	requires	59.99	5.49	12.72
	found	59.80	5.50	12.40

TABLE I

Base	Solvent	Temp of Addition of PhCOCl	Conditions	Yield of (6) (n.m.r.)%
NaH	Benzene	RT	4 hrs reflux	49
NaH	Benzene	80°C	4 hrs reflux	16
nBuLi	Dioxan	0°C	2 hrs reflux	17
$KOBu^t$	Benzene	0°C	30 mins RT	33
$PhCH_2\overset{+}{N}(Me)_3\bar{O}Me$	THF	0°C	incomplete reaction after 1 hr RT	mixture too complex to analyse

Reactions of 3-nitro-2-butanone with Benzamide

3-nitro-2-butanone (468 mg , 4 mM), benzamide (484 mg, 4 mM) and p-toluene sulphonic acid (19 mg, 0.1 mM) were dissolved in dry benzene and refluxed for 90 hrs, using a Dean-Stark water separator. The reaction was followed by t.l.c. (Silica gel/CH_2Cl_2). Solvent and some unreacted nitro ketone were removed in vacuo yielding brown semi-crystalline solid, which was dissolved in CH_2Cl_2 and the solution quickly washed with dil. Na_2CO_3, then

water. Drying and evaporation gave a brown solid (457 mg) which was recrystallised ($CHCl_3$), affording a first crop of benzamide (150 mg). The dark brown residue was chromatographed by p.l.c. (Silica gel,2 mm; 1 : 9 Pentane/CH_2Cl_2, eluting 2x). (6) was the fastest running fraction (Rf ~0.6) of at least five components. The crude product was recrystallised (EtOH) to yield pure (6), (50 mg, 5.7%) m.p. 108-109°C. A second fraction (Rf ~0.25) was identified as N-acetylbenzamide.

m.p.	:	117-118°C (lit.[109] 117°C)
n.m.r. ($CDCl_3$)	:	δ=2.62 (S, 3H), 7.40-7.70 (m, 3H) 7.88 (m, 2H), 8.85 (brS, 1H);
i.r. (CH_2Cl_2)	:	νcm^{-1} 3350 w, 1715 s, 1690;
m.s.	:	M^+ 163, B 105$(PhCO)^+$

Several experiments using modified conditions, e.g., refluxing in xylene as solvent, $KHSO_4$ as catalyst, or reacting benzamide directly with the nitroketone in glacial HOAc/NaOAc at 100-110°C, were unsatisfactory for preparing (6).

Attempted Preparation of β-alkoxy substituted nitro-olefins

2-methoxy-3-nitrobut-2-ene

3-nitro-2-butanone (234 mg, 2 mM) was dissolved in 1 N NaOH (2.2 ml, 2.2 mM) (soln. ca. pH 8) and MeOH (0.6 ml) added followed by redistilled dimethyl sulphate (252 mg, 2 mM), which made the solution acidic (pH 3). 1 N NaOH (0.2 ml) was added to readjust the pH to ca. 8, and the solution stirred at room temperature for 3½ hrs, observing the reaction by t.l.c. Removal of solvent followed by extraction with CH_2Cl_2 gave a colourless oil (18 mg) which was several components and could not be identified. The aqueous solution was acidified to pH 4 with dil. HOAc, then extracted with CH_2Cl_2, yielding a brown oil (3 mg) which was not identified. The remainder of the product material appeared to be very water soluble.

A similar reaction replacing dimethyl sulphate by methyliodide remained at alkaline pH after addition of the alkylating agent. After 3½ hrs, the mixture was still alkaline; removal of low boiling materials and examination of the mixture by n.m.r. only indicated the presence of unreacted enolate anion and nitroketone.

Formation of the enolate anion by reacting the nitroketone with a slight excess of NaH in DMSO, followed by addition of methyl iodide led to a mixture of products after 2 hrs. No 3-methoxy-2-nitrobut-2-ene could be identified in the mixture.

3,3-dimethoxy-2-nitrobutane

3-nitro-2-butanone (468 mg, 4 mM) was refluxed with selenium dioxide (1.78 g, 16 mM) in dry MeOH (40 ml). Samples were removed periodically, the solvent removed and the mixture examined by i.r. spectroscopy and t.l.c. After 45 hrs black selenium began to deposit, so reaction was ceased and the methanol evaporated, yielding a red-black suspension, which was chromatographed by silica gel filtration, eluting with CH_2Cl_2, then 2% MeOH/CH_2Cl_2. The first fractions contained a colourless crystalline compound (8 mg) which sublimed up the walls of the sample vial when stored in the refrigerator. It was assigned the nitronic acid structure (38) (1.7%)

m.p.	: 75-76°C		
n.m.r. ($CDCl_3$)	: δ=1.95 (S, 3H), 2.32 (S, 3H), 8.10 (brS, 1H)		
i.r. (CH_2Cl_2)	: ν cm^{-1} 3475 s, 3200 brm, 1680 s, 1620 w, 1350s		
u.v. (MeOH)	: 230 nm		
	%C	H	N
$C_4H_7NO_3$ requires	41.42	6.03	11.96
found	41.42	6.27	12.07

The second main fraction was a yellow oil (1.3 g) which deposited red crystals on standing. Further chromatography on silica gel, eluting with Et_2O, gave a brown oil (250 mg) which slowly precipitated more red crystals. It was found impossible to purify this material completely but its spectroscopic data suggested the dimethoxy nitronic acid structure (42)

n.m.r. ($CDCl_3$)	: δ=1.91 (S, 3H), 2.30 (S, 3H), 3.40 (S, 6H) 5.28 (brS, ?H)
i.r. (CH_2Cl_2)	: ν cm^{-1} 3620 w, 3500 ms, 3300-2900 br, 1680 s, 1600 m, 1350 s.

3,3-diethoxy-2-nitrobutane

A series of experiments were performed by reacting molar equivalents of triethylorthoformate and 3-nitro-2-butanone, in dry EtOH with various

catalysts at a level of about 0.1 equivalents. Catalysts examined were ammonium nitrate, hydrogen chloride, anhydrous ferric chloride, and p-toluene sulphonic acid. Temperatures of reaction ranged from room temperature to reflux, and reactions were continued for long periods ($6\frac{1}{2}$ days). Conditions were not found by which complete conversion could be effected, and after running reactions for long periods or high temperatures, complex mixtures of products resulted.

Ring A Carboxylic Acids

Preparation of Ethylene ketal ethyl ester (8)

Ethyl Hagemann's ester (18.2 g, 0.1 M), 1,2-ethanediol (6.8 g, 0.11 M) and p-toluene sulphonic acid (50 mg) were dissolved in benzene, and refluxed using a Dean-Stark distillation head until no more water formed ($6\frac{1}{2}$ hrs). The solution was cooled, neutralised with dilute NaOAc, washed with ice-cold water, then dried and evaporated. The pale yellow oil was distilled under reduced pressure (b.p. 100-106°/0.2 mm) giving a colourless liquid[110] (18.95 g, 84%).

n.m.r. ($CDCl_3$) : δ = 1.25 (t, 7, 3H), 1.47-1.82 (m, 2H), 1.97 (brS, 3H), 2.15-2.60 (m, 4H), 3.90 (S, 4H), 4.15 (q, 7, 2H)

i.r. (film) : $\nu\,cm^{-1}$ 2975 s, 2940 s, 2880 s, 1710 s, 1640 m

	%C	H
$C_{12}H_{18}O_4$ requires	63.70	8.02
found	63.79	8.15

Preparation of Ethylene ketal carboxylic acid (9)

The ketal ethyl ester (8) (2.26 g, 10 mM) was refluxed with 2 N NaOH (10 ml, 20 mM) until the solution was homogeneous (30 mins). The basic solution was extracted with Et_2O to remove neutral material (~35 mg). The aqueous solution was acidified with dilute H_2SO_4 then extracted with Et_2O yielding a brown oil which crystallised (1.85 g, 93%). A lower yield was

obtained after recrystallisation from CCl_4/60-80° petrol (-20°C) (800 mg).

m.p.	: 81-83°C
n.m.r. (CCl_4)	: δ = 1.65 (m, 2H), 2.06 (brS, 3H), 2.20-2.65 (m, 4H), 3.88 (S, 4H)
i.r. (CH_2Cl_2)	: ν cm^{-1} 3400 w, 2860 brs, 2600 brm, 1680 s, 1628 s

	%C	H
$C_{10}H_{14}O_4$ requires	60.59	7.12
found	60.71	7.24

Preparation of Ethylenethioketal ethyl ester (12)[88]

A mixture of ethyl Hagemann's ester (9.1 g, 50 mM) and ethanedithiol (9.4 g, 100 mM), in glacial HOAc, was cooled in ice-salt. Borontrifluoride diethyl etherate (2.8 ml, 22 mM) was added dropwise resulting in a stiff paste, which was stirred in ice-salt for 1 hr. The mixture was allowed to warm to 4°C, and stirring was continued for a further 20 hrs, after which the crude product was evaporated at room temperature in vacuo (0.3 mm). Water was added and the suspension extracted with Et_2O. Extracts were washed with dilute $NaHCO_3$, dried then evaporated to give a crude yellow oil, which was distilled in vacuo (b.p. 116-119°C/0.15 mm) to give a colourless viscous oil (8.1 g, 63%). In the latter stages of the distillation, the crude product became extremely viscous and distillation ceased, even though some thioketal was still present.

n.m.r. ($CDCl_3$)	: δ = 1.27 (t, 7, 3H), 1.74 (brS, 3H), 1.90-2.40 (m, 4H), 2.95 (m, 1H), 3.35 (S, 4H, 4.18 (q, 7, 2H), 5.76 (m, 1H)
i.r. (film)	: ν cm^{-1} 2970 ms, 2912 s, 2860 m, 1730 s, 1655 m

	%C	H	S
$C_{12}H_{18}O_2S_2$ requires	55.77	7.02	24.82
found	56.02	7.11	24.59

Preparation of Ethylenethioketal carboxylic acid (13)

Ethylenethioketal ethyl ester (12) (1.29 g, 5 mM) and NaOH (1.6 g, 40 mM) in a mixture of MeOH (25 ml) and water (10 ml) were refluxed so that a homogeneous solution resulted (1 hr), by which time the reaction mixture was brown. The reactants were refluxed a further 1 hr then cooled; the MeOH was evaporated,

and the aqueous solution extracted with Et_2O to remove neutral material (35 mg). The aqueous solution was cooled by addition of ice, followed by acidification to pH 3 with 1 N HCl. The off-white precipitate was extracted with $CHCl_3$ and extracts were washed with brine, water, then dried. Evaporation gave an orange oil which was redissolved in $CHCl_3$ and boiled with activated charcoal to decolourise, yielding a yellow oil (924 mg, 80%) which crystallised. Recrystallisation was from Et_2O/pentane yielding white crystals.

m.p. : 137-138°C

n.m.r.($CDCl_3$) : δ = 1.70 (brS, 3H), 1.60-2.40 (m, 4H), 2.82 (m, 1H), 3.35 (S, 4H), 5.82 (m, 1H), 11.72 (brS, 1H)

On standing, the signal at δ 5.82 (C = C<u>H</u>) diminished and was accompanied by a new resonance at 5.59 (m); total of two resonances = 1H. The remaining signals showed some broadening.

i.r. (CH_2Cl_2) : νcm^{-1} 3470 w, 3440-3040 br, 2925 s, 2875 s, 1740 sh, 1690 s, 1632 ms

	%C	H	S
$C_{10}H_{14}O_2S_2$ requires	52.14	6.13	27.84
found	51.75	6.29	27.62

<u>Preparation of Enolmethylether ethyl ester (15) (1-Ethoxycarbonyl-4-methoxycyclohexa-1,3-diene)</u>

Potassium t-butoxide (8.4 g, 75 mM) was dissolved in DMSO (80 ml) under N_2 and cooled in ice-water. Ethyl Hagemann's ester (13.65, 75 mM) was added in 5 mins, and the resulting brown solution stirred at room temperature for 15 mins, followed by cooling in ice-water. Dimethyl sulphate (9.4 g, 75 mM) was added, and the solution allowed to warm to room temperature. The reaction appeared to be over after 3 hrs, but was stirred for a total of 18 hrs at room temperature to ensure completion. The reaction mixture was poured on to ice (400 g), and extracted with Et_2O. Washing with water, drying and evaporation gave a yellow oil (11.9 g), which was 90% pure by g.l.c. (6' SE 30, 170°C), 73% yield. Fractional distillation <u>in vacuo</u> gave the pure ester (15) (b.p. 67-72°/0.1 mm)

n.m.r. ($CDCl_3$) : δ = 1.18 (t, 7, 3H), 1.70-2.70 (m, 4H), 2.2 (brS, 3H), 3.63 (S, 3H), 4.15 (t, 7, 2H), 4.93 (S, 1H)

i.r. (film) : νcm^{-1} 2980 ms, 2940 ms, 2900 m, 2935 m, 1728 s, 1687 s, 1642 s, 1563 s

m.s. : m/e M^+ 196, B 123

Accurate mass M^+ 196.1094, $C_{11}H_{16}O_3$ requires M^+ 196.1099

Preparation of Enolmethylether carboxylic acid (16) (4-Methoxy cyclohexa-1,3-diene-1-carboxylic acid)

The enolmethylether ester (15) (980 mg, 5 mM) and NaOH (800 mg, 20 mM) were refluxed in MeOH (5 ml) and water (10 ml) for 4 hrs, requiring $2\frac{1}{2}$ hrs for the mixture to become homogeneous. MeOH was removed in vacuo and neutral material extracted with Et_2O. The aqueous solution was acidified with 1 N HCl to pH 3, precipitating an off-white solid which was extracted with CH_2Cl_2. Washing, drying, and evaporation gave a light brown crystalline solid (605 mg, 72%) which was one component by t.l.c., and was recrystallised from CCl_4/40-60° petrol yielding white crystals.

m.p. : 100-104°

n.m.r.($CDCl_3$) : δ = 1.75-2.75 (m, 4H), 2.25 (brS, 3H), 3.68 (S, 3H), 5.01 (S, 1H), 12.15 (brS, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3500 w, 3360-3040 br, 2938 m, 2840 m, 1663 s, 1550 s

m.s. : m/e M^+ 168, B 123

Accurate mass M^+ 168.1779; $C_9H_{12}O_3$ requires M^+ 168.0786

Preparation of Dimethylketal ethyl ester (19)

Ethyl Hagemann's ester (3.64 g, 20 mM) and trimethylorthoformate (3.18 g, 30 mM) were dissolved in MeOH (25 ml). p-Toluene sulphonic acid (190 mg, 1 mM) was added causing the solution to turn dark green. The solution was stirred for 12 hrs at room temperature, then dilute $NaHCO_3$ was added to neutralise. MeOH was evaporated and the mixture extracted with CH_2Cl_2, followed by washing of extracts with saturated brine and water. Drying and evaporation yielded a brown oil (4.2 g, 89% crude) whose spectroscopic data were satisfactory for the ester (19), but the material was also contaminated

with about 10% of the enol ether (15) formed by elimination of MeOH from the ketal. The ketal was difficult to purify and was not distilled, since a similar reaction carried out in refluxing methanol gave a much greater proportion of (15) in the product. It was feared that distillation might cause elimination of MeOH from the ketal, so the crude product was used directly.

n.m.r. ($CDCl_3$) : δ = 1.30 (t, 7, 3H), 1.80 (m, 2H), 2.04 (brS, 3H), 2.15-2.67 (m, 4H), 3.23 (S, 6H), 4.20 (q, 7, 2H)

i.r. (film) : νcm^{-1} 2980 ms, 2950 s, 2910 ms, 2818 m, 1730 shs 1709 s, 1642 m, 1568 m

m.s. : m/e M^+ 228, B 88

Accurate mass M^+ 228.1368; $C_{12}H_{20}O_4$ requires M^+ 228.1361

Preparation of Dimethyl ketal carboxylic acid (20)

The crude dimethyl ketal ester (19) (1.15 g ~5 mM) and NaOH (400 mg, 10 mM) in water (5 ml) were refluxed in dioxan (30 ml) but the mixture failed to homogenise after 20 hrs and became dark brown. The mixture was stood for a further 40 hrs at room temperature then the dioxan was removed in vacuo and the residue diluted with water, and extracted with ether to remove neutral material. The aqueous solution was cooled in ice and acidified to pH 4 with 1 N HCl giving an emulsion. NaCl was added to help break the emulsion and the product was extracted with CH_2Cl_2, but the extraction was made difficult by the presence of what appeared to be polymeric material. Extracts were washed with saturated brine, water, then dried and evaporated. The product a brown oil (215 mg), crystallised with difficulty from Et_2O/pentane (-20°C) to give oily crystals which were recrystallised from Et_2O/pentane (-20°C). An experiment using methanol as the solvent failed to improve the hydrolysis, and conditions were not found for optimising this reaction.

m.p. : 111-113°C

n.m.r. ($CDCl_3$) : δ = 1.85 (m, 2H), 2.10 (brS, 3H), 2.15-2.70 (m, 4H) 3.25 (S, 6H), 10.20 (brS, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3450-3080 br, 2950 s, 2818 ms, 1690 brS, 1637 m, 1560 m

m.s. : m/e M^+ 200, B 88

Accurate mass M^+ 200.1034; $C_{10}H_{16}O_4$ requires M^+ 200.1048

Attempted preparation of Ring A Acid Chlorides

Reagents

Thionylchloride was distilled from quinoline, collecting a mid-fraction b.p. 76°, which was faintly yellow. Triphenylphosphine was recrystallised from hexane. HMPA was stirred with CaH_2 and distilled under reduced pressure. DMF was stirred with BaO, decanted, then distilled in vacuo. Benzene was distilled and dried over sodium wires. $CHCl_3$ was washed with water, dried, and distilled from P_2O_5. CCl_4 was distilled from P_2O_5.

Ethyleneketal acid chloride (10)

Thionylchloride

Reactions were followed by observing the carbonyl region of the i.r. spectrum of the mixtures. When reactions were terminated, the n.m.r. spectrum was recorded. Conditions were not found whereby the pure acid chloride (10) could be prepared using thionylchloride; extensive decomposition of products occurred whether using the acid (9) or the sodium salt (9a). Reaction conditions are summarised in Table 2; all were performed under N_2.

TABLE 2

	Ring A Precursor	Equivalents $SOCl_2$.	Conditions
(1)	(9)	20	In benzene, RT, 24 hrs.
(2)	(9)	20	3 hrs RT, ½ hr reflux, no solvent.
(3)	(9)	20	1½ hrs reflux, no solvent.
(4)	(9a)	1.05	In $CHCl_3$, 0.1 equivalent DMF, reflux 1 hr.
(5)	(9)	(1.05 + 1.0 DMF)	Mixed with [(9) + 1 equivalent DMF][82] in $CHCl_3$, then refluxed 1½ hrs.
(6)	(9a)	(1.05 + 1.0 DMF)	As (5); refluxed 3 hrs.
(7)	(9)	3.0	In HMPA 10 mins RT, 1 hr 70°C.
(8)	(9)	1.0	In HMPA (-20°C)[83], then warmed to RT 1 hr.

Phosphorus pentachloride

Reactions were carried out in ethanol free $CHCl_3$ or $CDCl_3$, monitoring by i.r. or n.m.r. Opening of the ethylene ketal, and extensive decomposition occurred in all experiments; some polymeric material was also formed.

TABLE 3

Ring A Precursor	Equivalents PCl_5	Conditions
(9)	1.1	In $CHCl_3$ at RT, with N_2 to expel HCl
(9)	1.0	In $CDCl_3$, 37°C

Ph_3P/CCl_4 [86]

The acid (9) was reacted at 37°C with Ph_3P (1 equivalent) in CCl_4, monitoring the reaction by n.m.r. No reaction occurred after 1 hr, so the mixture was refluxed for 3 hrs by which time all the acid reacted. The infra red spectrum showed no acid chloride.

Thioketal acid chloride (14)

The thioketal acid (13) was mixed with 5 equivalents of thionyl chloride at room temperature, resulting in vigorous effervescence and a blue-green mixture which rapidly turned black. After 2 hrs the spectroscopic data was too ill resolved to be completely interpretable although there was no thioketal group in the n.m.r. spectrum of the product. The acid chloride could not be identified from the i.r. spectrum.

In a similar reaction in $CDCl_3$ (0.5 M soln. of acid), monitored by n.m.r., after 5 min at 37°C decomposition began to occur and was complete after 75 mins. The product was unidentifiable.

Enolmethylether acid chloride (17)

1. Thionyl chloride (5 equivalents) was added to the acid (16) in dry benzene causing rapid effervescence. The solution blackened and a brown precipitate formed after $2\frac{1}{2}$ hrs the i.r. spectrum showed several carbonyl stretching frequencies; some polymeric material was formed and the n.m.r. spectrum was ill resolved.

2. A similar reaction was carried out using thionyl chloride and the sodium salt of (16). After 3 hrs at room temperature a brown mixture resulted which was a mixture of products and had similar spectroscopic properties to the product from the free acid and thionyl chloride.

3. The acid (16) in CCl_4 (0.4 M solution) was reacted with Ph_3P (1 equivalent) and the reaction at 37°C was monitored by n.m.r. Reaction was slow, and after 23 hrs the acid had all reacted, but on removal of solvent after 30 hrs no acid chloride could be identified.

Preparation of 3, 3-dimethylacryloyl chloride (22)

3, 3-dimethylacrylic acid (21) was reacted with thionyl chloride according to Auwers[84] to give the pure acid chloride.

n.m.r. ($CDCl_3$)	:	δ = 1.96 (d, 1.5, 3H), 2.24 (d, 1.5, 3H) 6.00 (m, 1H)
i.r. (film)	:	νcm^{-1} 2980 w, 2945 w, 2915 w, 1783 s, 1750 s, 1660 w, 1617 s

Reaction of 3, 3-dimethylacrylol chloride with (5a)

A pre-prepared sample of the lithium salt of 3-amino-2-nitrobut-2-ene (0.5 mM) was suspended in dry benzene (0.5 ml) and dimethylacryloyl chloride (22) (59 mg, 0.5 mM) was added. Reaction was slow and stirring was continued for 9 hrs at room temperature under N_2. The suspension was diluted with CH_2Cl_2 then filtered through celite. Evaporation of the filtrate gave a semi-crystalline yellow solid (50 mg), which contained the expected amide product (23), 3-amino-2-nitrobut-2-ene and three other unidentified compounds. Separation was by p.l.c. (Silica gel, 0.75 mm, eluting with CH_2Cl_2; Rf ~0.6) to give pure amide (23) 6 mg, 6%).

n.m.r. ($CDCl_3$)	:	δ = 1.95 (d, 1.5, 3H), 2.21 (brS, 6H), 2.60 (S, 3H), 5.79 (m, 1H). NH not observable.
i.r. (CH_2Cl_2)	:	νcm^{-1} 3660 w, 3400 br, 2910 w, 1700 ms, 1640 s, 1603 s
m.s.	:	m/e M^+ 198, B 83 $((CH_3)_2C = CHCO)^+$ Accurate mass M^+ 198.1011; $C_9H_{14}N_2O_3$ requires M^+ 198.1004

Amide derivatives of ring A precursor (9)

Preparation of Ethylene ketal cyanomethyl ester (24)

Lithium ethoxide (10.8 ml of a 0.46 M soln. in EtOH, 5 mM) was added to the acid (9) (990 mg, 5 mM) in EtOH, and the solution stirred for 15 mins then the solvent removed in vacuo. The resulting crystalline solid was dissolved in dry DMSO (5 ml), chloroacetonitrile (1.51 g, 20 mM) was added, and the solution stirred at room temperature. After 1 hr the solution was

neutral; after 3 hrs ice-water was added and the product extracted with Et_2O. Drying and evaporation in vacuo gave a brown oil (990 mg) which was pure by n.m.r. (~84% yield). The product was distilled under reduced pressure to give a colourless oil (b.p. 98°C/0.015 mm). The product was extremely viscous and ceased to distil in the latter stages, even though the bath temperature was ca. 200°C.

n.m.r. ($CDCl_3$) : δ = 1.60-1.90 (m, 2H), 2.08 (brS, 3H), 2.30-2.72 (m, 4H), 3.96 (S, 4H), 4.74 (S, 3H)

i.r. (film) : ν cm^{-1} 2958 m, 2890 m, 1722 s, 1638 ms

		%C	H	N
$C_{12}H_{15}NO_4$	requires	60.75	6.37	5.90
	found	60.60	6.34	6.06

Reactions of Cyanomethyl ester (24)

With Benzylamine

Heating molar equivalents of the cyanomethyl ester (24) and benzylamine in several solvents: $CDCl_3$ (20 hrs, reflux), DMSO (24 hrs, 70°C); or direct fusion (40 hrs, 70°C) failed to produce any detectable reaction.

With salts of 3-amino-2-nitro-but-2-ene (5a)

The lithium salt of 3-amino-2-nitrobut-2-ene (0.4 mM) in DMSO (1 ml) did not react at all when stirred with a molar equivalent of the cyanomethyl ester (24) at room temperature for 3 hrs.

Acyloxophosphonium derivatives[90,91]

(1) With Benzylamine

Ph_3P (131 mg, 0.5 mM) dissolved in CCl_4 (0.5 ml) and THF (0.15 ml) was refluxed 30 mins forming a yellow solution, and a little brown solid material. The mixture was cooled in ice and the acid (9) (99 mg, 5 mM) was added. The then colourless solution was stood in ice for 10 mins. Benzylamine (107 mg, 1 mM) was added and the resulting white suspension was refluxed 30 mins. Dilution with ether, followed by filtration and evaporation gave a yellow oil (245 mg). This contained the desired benzamide derivative (27) and triphenylphosphine oxide, which was particularly difficult to remove, as it co-crystallised with the amide and had a similar Rf on t.l.c.

n.m.r. ($CDCl_3$) : δ = 1.60-2.00 (m, 2H), 1.77 (brS, 3H), 2.15-2.65 (m, 4H), 3.90 (S, 4H), 4.48 (d, 7, 2H) 5.78 (brS, 1H). Aromatic region contaminated with Ph_3PO.

(2) With salts of 3-amino-2-nitrobut-2-ene (5a)

The acyloxophosphonium intermediate from acid (9) was prepared as above on a 1 mM scale, followed by addition of the sodium salt of (5a). Refluxing for 30 mins gave a dark brown mixture. Addition of water and extraction with CH_2Cl_2 gave a viscous oil (396 mg) which contained 3-amino-2-nitrobutene. There was no amide present. Acidification of the aqueous solution with dilute HCl, followed by extraction gave a brown oil (200 mg) which was mainly acid (9), but also contained 3-nitro-2-butanone arising from hydrolysis of (5).

Amides via DCC Intermediates

Reaction of Benzylamine with ethylene ketal acid (9)

The acid (9) (99 mg, 0.5 mM) was dissolved in CH_2Cl_2 (0.5 ml) with DCC (103 mg, 0.5 mM), followed by rapid* addition of benzylamine. The suspension was stirred for $7\frac{1}{2}$ hrs at room temperature, then filtered washing the residue (40 mg) with CH_2Cl_2. The filtrate was evaporated giving a crude semi-solid to which ether was added causing further precipitation. The suspension was filtered, and the filtrate evaporated to give a viscous oil containing the desired amide (27) (Rf ~ 0.1 Sigel; 0.5% MeOH/$CHCl_3$). The crude material was chromatographed on silica gel (p.l.c. grade eluting first with 0.5% MeOH/$CHCl_3$ to remove some fast running material (39 mg), then progressively increasing to 5% MeOH/$CHCl_3$ whereby the amide (27) (97 mg, 77% crude) was obtained as a yellow oil. This material failed to crystallise after repeated attempts but had satisfactory spectroscopic properties.

n.m.r. ($CDCl_3$) : δ = 1.60-2.00 (m, 2H), 1.77 (brS, 3H), 2.15-2.65 (m, 4H), 3.90 (s, 4H), 4.48 (d, 7, 2H), 5.78 (brS, 1H), 7.21 (brS, 5H)

i.r. (CH_2Cl_2) : νcm^{-1} 3430 ms, 3025 m, 2945 ms, 2910 ms, 2885 ms, 1648 brs, 1546 m, 1500 s

m.s. : m/e M^+ 287 B 86

Accurate mass M^+ 287.1513; $C_{17}H_{21}NO_3$ requires M^+ 287.1521.

*N.B. If addition of benzylamine was delayed, then transacylation occurred in the DCC intermediate, forming a very insoluble material.

Reactions of Aryl Sulphonyl Chlorides

Using Dimethylacrylic acid (21)

A series of model experiments were carried out using dimethylacrylic acid and aryl sulphonyl chlorides (p-toluene sulphonyl chloride (pTSC), and 2,4,6-tri-isopropyl sulphonyl chloride (TPS)) with various bases. The value of the intermediate sulphonic anhydride produced was judged by its reaction with benzylamine. Conditions examined are listed as follows. All reactions were followed by i.r. spectroscopy, and none proceeded to completion.

(1) (21) + pTSC (1 equiv.) + N,N-dimethylaminopyridine (1 equiv.) in CH_2Cl_2 : reached equilibrium after 30 mins - ran for 3 hrs.

(2) (21) + pTSC (1 equiv.) + pyridine (6.5 equiv.) : reached equilibrium after 30 mins - ran for 2 hrs.

(3) (21) + TPS (1 equiv.) + pyridine (13 equiv.) : ran 23 hrs.

(4) Lithium salt of (21) + TPS (1 equiv.) in CH_3CN : ran 20 hrs.

Benzylamine (1 equiv.) was added to each of the reactions, in all cases forming a mixture of the amide (34), and a sulphonamide (e.g. (35)). For example, in experiment (1), (34) and (35) were in the ratio 1 : 1.2 . The sulphonamide could be preferentially crystallised (CCl_4) leaving the amide (34) in the residue. Attempts to crystallise this material failed, although slight crystallisation occurred after storage in the refrigerator for 6 months.

n.m.r. ($CDCl_3$) : δ = 1.75 (S, 3H), 2.08 (S, 3H), 4.26 (d, 7, 2H)
5.55 (m, 1H), 6.50 (brS, 1H), 7.18 (S, 5H)

Using Ethylene ketal acid (9)

The acid (9) (99 mg, 0.5 mM) and TPS (302 mg, 1 mM) were dissolved in pyridine (1 ml) and stirred at room temperature for 3 hrs, following the reaction by i.r. (in a separate experiment, warming to 100°C decomposed the sulphonic anhydride that had already formed). Benzylamine (53.5 mg, 0.5 mM) was added, forming a yellow solution and a fine white precipitate. The suspension was stirred at room temperature for $6\frac{1}{2}$ hrs, then ice-water was added and the solution acidified with 1 N HCl. Extracting with Et_2O, drying and evaporation gave a brown oil (173 mg) which contained TPS, the amide (27), some sulphonamide and several other components. The amide (27) was separated by chromatography

on silica gel eluting first with CH_2Cl_2, then 5% MeOH CH_2Cl_2, and was isolated in low yield (15 mg), but had the same spectroscopic properties to the material isolated earlier.

Mixed carboxylic acid anhydride method

Reaction of Dimethylacrylic acid with Benzylamine

Dimethylacrylic acid (100 mg, 1 mM) and triethylamine (101 mg, 1 mM) were dissolved in CH_2Cl_2 (1.5 ml), and cooled to 0°C. Pivaloyl chloride (120.5 mg, 1 mM) was added forming a white precipitate, and stirring was continued for 2 hrs at 0-5°C. Benzylamine (107 mg, 1 mM) was added and the suspension stirred for 12 hrs. Water was added and the solution extracted with CH_2Cl_2. The extracts were washed with water, dried and evaporated yielding a colourless oil (210 mg) containing 61% (n.m.r.) amide (34) (67% yield). This material crystallised with some difficulty from Et_2O/pentane (-78°C) to give oily crystals which were not purified further. The spectroscopic properties were consistent with structure (34).

n.m.r. ($CDCl_3$)	:	δ = 1.78 (S, 3H), 2.08 (S, 3H), 4.28 (d, 7, 2H) 5.55 (m, 1H), 6.55 (brS, 1H), 7.18 (S, 5H)
i.r. (CH_2Cl_2)	:	ν cm^{-1} 3439 m, 3030 w, 2960 m, 2918 m, 2875 m, 1668 s, 1647 s, 1503 s
m.s.	:	m/e M^+ 189, B 83 $((CH_3)_2C{=}CHCO)^+$ Accurate mass M^+ 189.1153; $C_{12}H_{15}NO$ requires M^+ 189.1161

Reaction of Dimethylacrylic acid with a salt of 3-amino-2-nitrobut-2-ene (5a)

The mixed anhydride from dimethylacrilic acid and pivaloyl chloride was prepared in CH_2Cl_2 as described, and the suspension was added to the lithium salt of 3-amino-2-nitrobut-2-ene (1 equivalent) at 0°C. Stirring was continued at room temperature but there was no reaction after 15 hrs. An identical reaction carried out in CH_3CN gave no amide after 72 hrs. Evaporation and work up with water, followed by extraction into CH_2Cl_2 gave a brown crystalline solid which was predominantly 3-amino-2-nitrobut-2-ene. No amide (23) could be identified.

Hydrazine Derivatives

Reaction of Hydrazine Hydrate with 3-nitro-2-butanone

Hydrazine hydrate (200 mg, 4 mM) was added to 3-nitro-2-butanone (468 mg, 4 mM) in an n.m.r. tube, causing a vigorous reaction and two layers separated. The mixture was basic (pH 9-10). N.m.r. indicated only nitroethane and N-acetylated material. Shaking with $CDCl_3$ and re-recording the n.m.r. spectrum extracted only nitroethane (δ 1.55 (t, 7.), 4.40 (q, 7)). In two separate experiments adding 1.0 and 1.5 equivalents of glacial HOAc to the nitroketone prior to the addition of hydrazine, nitroethane was again the major product.

Preparation of Benzhydrazide (46)

Benzhydrazide was prepared from benzoyl chloride and hydrazine hydrate as described[105].

m.p.	:	112°C ($CHCl_3$) lit.[105] 112.5°C
n.m.r. ($CDCl_3$)	:	δ = 4.1 (brS, 3H), 7.4-7.68 (m, 3H), 7.68-8.1 (m, 2H)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3675 w, 3435 m, 3320 w, 1670 s, 1628 ms, 1580 m, 1502 m

Reactions of Benzhydrazide with 3-nitro-2-butanone

N.m.r. observations indicated that mixing molar equivalents of benzhydrazide with 3-nitro-2-butanone resulted in the immediate liberation of nitroethane. This was prevented,by carrying out the reaction under acidic conditions.

Benzhydrazide (418 mg, 3 mM) and glacial HOAc (180 mg, 3 mM) were mixed with benzene (5 ml). 3-nitro-2-butanone (351 mg, 3 mM) was added and the suspension was refluxed for 1 hr. Benzene (25 ml) was added and the water formed in the reaction was removed as a benzene azeotrope by distillation into a Dean and Stark water separator during 4 hrs. Solvent was removed *in vacuo* and the residue evaporated under vacuum (0.1 mm) to remove traces of HOAc. The crude product was recrystallised from benzene (310 mg, 42%); evaporation, then crystallisation from Et_2O gave a second crop of white crystals (47) (340 mg, 46%, total 88%).

m.p.	:	96-98°C
n.m.r.	:	δ = 1.68 (d, 7, 3H, $\underline{CH_3}CHNO_2$), 2.05 (S, 3H), 5.28 (q, 7, 1H, $CH_3\underline{CH}NO_2$), 7.30-7.65 (m, 3H), 7.65-2.00 (m, 2H), 9.28 (brS, 1H)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3650 w, 3475 w, 3425 w, 1690 brs, 1630 m, 1603 w, 1552 s, 1510 ms
u.v. (MeOH)	:	208, 232, 253 nm
m.s.	:	m/e M^+ 235, 189 $(PhCONHN{=}C(CH_3)CH(CH_3))^+$ Accurate mass 189.1035, $C_{11}H_{13}N_2O$ requires 189.1028, 161 $(PhCONHN{=}CCH_3)^+$ Accurate mass 161.0710, $C_9H_9N_2O$ requires 161.0715, 105 (B) $(PhCO)^+$

Preparation of Ring A hydrazine derivatives

Reaction of Ethylene ketal ester (8) with hydrazine

The ethylene ketal ester (8) (1.13 g, 5 mM) was heated at 100°C with hydrazine hydrate (1.0 g, 20 mM) using a procedure described for the preparation[104] of hydrazides. After 48 hrs no hydrazide was formed and the mixture contained at least seven components.

Reaction of Ethylene ketal cyanomethyl ester (24) with hydrazine

Refluxing the cyanomethyl ester (24) (58 mg, 0.25 mM) with hydrazine hydrate (23 mg, 0.25 mM) in CH_3CN for 48 hrs, gave a material containing many components, and the desired hydrazide could not be identified.

Preparation of Ethylene ketal hydrazide (45)

A mixture of the ethylene ketal acid (9) (495 mg, 2.5 mM) and triethylamine (300 mg, 2.5 mM) in dry CH_3CN (5 ml) were cooled to 0°C. Ethylchloroformate (270 mg, 2.5 mM) was added and the white suspension stirred for 30 mins at 0°C, followed by addition of hydrazine hydrate (125 mg, 2.5 mM). Stirring was continued for 1 hr at 0°C, then Et_2O (3 ml) was added and the suspension filtered to remove triethylamine hydrochloride. Evaporation and addition of Et_2O crystallised the hydrazide and filtration removed the regenerated acid (9) which was soluble in Et_2O. (The product was very soluble in water, and was extremely difficult to extract if aqueous workup conditions were used.) Traces of triethylamine hydrochloride were removed

by chromatography (Silica gel; 20% MeOH/CH_2Cl_2). The hydrazide (265 mg, 50%) was best recrystallised by dissolving in the minimum CH_2Cl_2, adding Et_2O to a point where turbidity was just observed, then cooling to give white needles.

m.p. : 109-109.5°C

n.m.r. ($CDCl_3$) : δ = 1.65-2.00 (m, 2H), 1.78 (brS, 3H), 2.18-2.70 (m, 4H), 3.98 (S, 4H), 7.17 (brS, 1H)

i.r. (CH_2Cl_2) : νcm^{-1} 3640 w, 3425 ms, 3320 w, 2950 ms, 2915 ms, 2885 ms, 1657 s, 1620 s

m.s. : m/e M^+ 212, 211 (M^+-H) Accurate mass 211.1073, $C_{10}H_{15}N_2O_3$ requires 211.1082, 181 (M^+-$NHNH_2$) Accurate mass 181.0850, $C_{10}H_{13}O_3$ requires 181.0864, 67 (B)

	%C	H	N
$C_{10}H_{16}N_2O_3$ requires	56.59	7.60	13.20
found	56.34	7.42	13.05

Preparation of the Ethylene ketal nitro hydrazide (49)

The ethylene ketal hydrazide (45) (350 mg, 1.64 mM) and glacial HOAc (98.5 mg, 1.64 mM) were mixed in benzene (5 ml). 3-nitro-2-butanone (192 mg, 1.64 mM) was added and the mixture refluxed using a Dean and Stark water separator. Water began to collect within minutes, and after 1 hr, benzene (40 ml) was added and refluxing was continued. The trap was drained periodically until ca. 30 ml were collected, after which no starting materials were present. The remaining solvent was removed in vacuo and the residue evaporated in high vacuum (0.1 mm). The crude product, a yellow oil, crystallised from Et_2O/pentane giving off-white crystals which were one component by t.l.c. (480 mg, 94%). Recrystallisation was from CH_2Cl_2 (min)/Et_2O as described for (45) to yield white crystals.

m.p.	:	106-107°C
n.m.r. ($CDCl_3$)	:	δ = 1.60-1.90 (m, containing 1.72 (d, 7) and 1.80 (brS) total 8H), 1.99 (S, 3H), 2.18-2.73 (m, 4H) 3.98 (S, 4H), 5.25 (m, 1H $CH_3C\underline{H}NO_2$)
90 MHz ($CDCl_3$)	:	δ = 1.53-1.90 (m containing 1.73 (d, 7) and 1.78 (brS), total 8H), 1.96 (S, 3H), 2.16-2.70 (m, 4H), 3.97 (S, 4H), 5.20 (m, 1H), 8.39 (brS, ½H), 8.91 (brS, ½H)
$(CD_3)_2SO$)	:	δ = 1.50-1.85 (m, containing 1.64 (d, 7) and 1.68 (brS), total 8H), 1.96 (S, 3H), 2.10-2.60 (m, 4H), 3.96 (S, 4H), 5.48 (q, 7, 1H, $CH_3C\underline{H}NO_2$)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3450 w, 2960 m, 2920 m, 2990 m, 1690s 1670 shs, 1552 s (NO_2)
u.v. (MeOH)	:	235 nm
(MeOH/NaOH)	:	233, 310, 400 nm
m.s.	:	m/e M^+ 311, 86 (B)
		Accurate mass M^+ 311.1469, $C_{14}H_{21}N_3O_5$ requires M^+ 311.1481

	%C	H	N
$C_{14}H_{21}N_3O_5$ requires	54.01	6.80	13.50
found	53.68	6.86	13.48

Reactions of Ethylene ketal nitrohydrazide (49)

(1) With aqueous acid - attempted ketal removal

(a) To a solution of the ketal nitrohydrazide (49) (104 mg, 0.33 mM) in dioxan (0.3 ml) and water (0.5 ml), was added 36% HCl (50 µl, ~0.5 mM) and the solution refluxed for 1 hr. By this time, t.l.c. (Silica gel 2% MeOH/CH_2Cl_2) indicated several products from which 3-nitro-2-butanone could be identified. Dioxan was evaporated in vacuo and the residue diluted with water and extracted with CH_2Cl_2. A low yield of product, a dark oil of several components was recovered. There was no ethylene ketal group in the n.m.r. of the product, but peaks corresponding to 3-nitro-2-butanone (δ 1.73 (d, 7), 5.32 (q, 7)) were present. A weak signal at δ 5.89 (m), characteristic of an α-proton in an enone system, was also observed.

i.r. (CH_2Cl_2) of crude mixture	:	νcm^{-1} 3450 m, 2920 ms, 1715 ms, 1688 ms, 1660 s, 1552 s

(b) The ethylene ketal nitrohydrazide (49) (104 mg, 0.33 mM) was dissolved in dioxan (0.5 ml) and 1N HCl (0.4 ml, 0.4 mM). The solution was stirred at room temperature and monitored by t.l.c. (Silica gel; 2% MeOH/CH_2Cl_2). Although 3-nitro-2-butanone was detected within 5 mins, 9 days were required before all the starting material disappeared. Water and dioxan were removed in vacuo to yield a brown gum which crystallised from Et_2O. Filtering off the solid (56 mg), and evaporation of the filtrate gave a brown oil (10 mg) which n.m.r. confirmed contained 3-nitro-2-butanone. The solid product was chromatographed on silica gel (20% MeOH/CH_2Cl_2) to give a yellow gum (30 mg) which crystallised from Et_2O. Its spectroscopic properties were the same as the ethylene ketal hydrazide (45), there was no evidence to suggest any deketalised product.

Reactions in DMSO

(a) Heating at 125°C

The ethylene ketal hydrazide (49) (50 mg, 0.16 mM) was heated at 125°C in $^2H_6$-DMSO in an n.m.r. tube occasionally cooling the sample and recording the n.m.r. spectrum. The quartet at δ 5.48 slowly diminished and had disappeared after 30 mins by which time the solution was black. A new sharp singlet was present at δ 3.45. The cooled solution was diluted with ice-water and extracted well with Et_2O. Washing, drying and evaporation of extracts gave a yellow oil (25 mg) which crystallised from Et_2O. Recrystallisation was from CH_2Cl_2 (min)/Et_2O as described for compound (45); the material was not analysed. The n.m.r. spectrum was badly resolved.

m.p.	:	172-174°C
n.m.r. ($CDCl_3$)	:	δ = 1.55-2.18 (m), 1.98 (S), 2.05 (S), 2.10-2.65 (m), 4.00 (S), 9.00 (brS)
i.r. (CH_2Cl_2)	:	νcm^{-1} 3545 m, 3420 w, 2950 ms, 2925 ms, 2885 ms, 1685 brs, 1610 m, 1552 w
m.s.	:	m/e highest ion 295 (1) (M^+-CH_4). Accurate mass 295.1159, $C_{13}H_{17}N_3O_5$ requires 295.1168,

237 (13) (295-CNO_2) Accurate mass 237.1231, $C_{12}H_{17}N_2O_3$ requires 237.1239, 181 (38), 99 (50),86 (69), 67 (B)

(b) Heating 190°C

A solution of (49) (31 mg, 0.1 mM) in $^2H_6$-DMSO (0.4 ml) was heated to reflux in an n.m.r. tube, and the n.m.r. spectrum of the mixture was recorded after 5 mins, then every 15 mins. Deketalisation began within 5 mins and was complete after 1 hr. DMSO was removed by evaporation in high vacuum (~0.1 mm) to give a black residue (25 mg) which was eluted through silica gel (p.l.c. grade) with 1% MeOH/CH_2Cl_2. Much black material was removed by this treatment but the product,a dark oil (11 mg),was several spots by t.l.c. The n.m.r. spectrum was complex and could not be resolved although a signal at δ 6.08 characterised the presence of an enone structure, perhaps (53).

n.m.r. ($CDCl_3$) : δ = 1.80-2.30 (m, ?), 2.30-2.80 (m, ?), 6.08 (m, 1H ?) and others

i.r. (CH_2Cl_2) : νcm^{-1} 3600 vbr, 2930 w, 1673 brs, 1605 w

(c) With catalytic CF_3CO_2H in DMSO

Trifluoroacetic acid (3 mg, 0.03 mM) was added to a solution of (49) (31 mg, 0.1 mM) in $^2H_6$-DMSO (0.4 ml) and the mixture stood at 37°C, periodically recording the n.m.r. spectrum. The quartet at δ5.48 very slowly decreased in intensity but was still present after 24 hrs. The sample was heated to 190°C and turned black within minutes. After 10 mins the mixture was cooled. The n.m.r. spectrum showed complete loss of the ethylene ketal signal, being replaced by a singlet at δ3.45. The quartet δ 5.48 had also disappeared and new signals corresponding to 3-nitro-2-butanone were observed.

Reaction of (49) with catalytic methoxide ion

To a solution of (49) (5 mg, 0.016 mM) in $^2H_4$-methanol (0.1 ml) was added $NaOCD_3$ (1 μl of a 4 M soln. in $^2H_4$-methanol, 0.004 mM). The then yellow solution was diluted to (0.3 ml) and the n.m.r. spectrum recorded at intervals. At 15 mins the nitro-methine proton could not be observed and

all the signals in the spectrum were broadened including the solvent peaks and TMS. After 45 mins the yellow colour faded but no change occurred in the spectrum. The sample was heated in an oil bath at 80°C (the pressure cap prevented violent boiling) for 2 hrs. No change occurred in the n.m.r. spectrum. The solution was stood at room temperature for 72 hrs and other than some changes in line shape, no new peaks formed, and it was clear that cyclisation was not occurring.

n.m.r. 90 MHz ($CDCl_3$) : δ = 1.55-1.87 (brm), 1.97 (S, 3H), 2.10-2.5 (brm), 3.98 (S, 4H)

Reactions of ethylene ketals with DMSO

1 : 4-dioxaspiro[4,5]decane (cyclohexanone ethylene ketal)

Cyclohexanone ethylene ketal was prepared from cyclohexanone and ethane diol and distilled under reduced pressure (b.p. 72-76°/15 mm; lit.[111] 174-180°/760 mm.

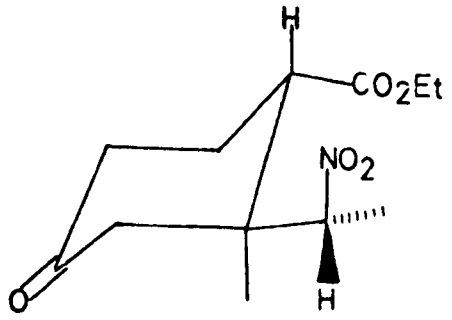
A solution of cyclohexanone ethylene ketal (14.2 mg 0.1 mM) in $^2H_6$-DMSO (0.4 ml) was heated to 190°C, monitoring changes by recording the n.m.r. spectrum at intervals. After 30 mins a new signal appeared at δ 3.45, but only marginally increased in intensity after a further 2 hrs. The remainder of the spectrum had broadened by this time.

In a similar experiment incorporating a 0.1 molar equivalent of anhydrous lithium perchlorate, deketalisation occurred at slightly slower rate than above.

Ethylene ketal ester (8)

After heating a solution of the ethylene ketal ester (8) (22.6 mg, 0.1 mM) in $^2H_6$-DMSO at 190°C, recording the n.m.r. spectrum periodically, no changes occurred after 2 hrs.

CHAPTER IV



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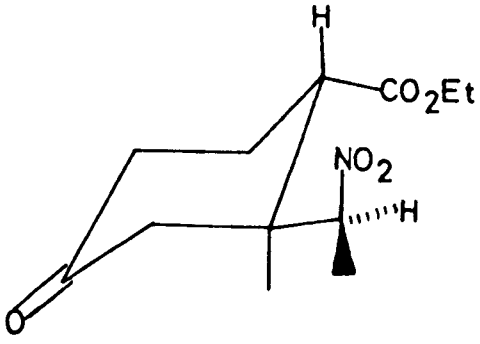

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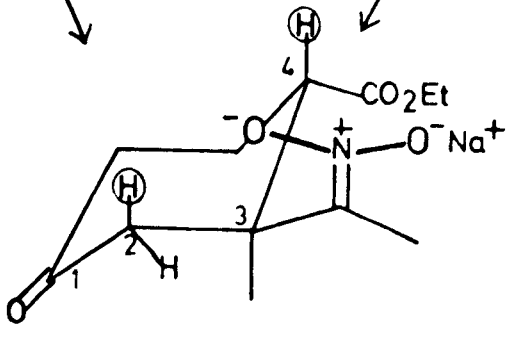

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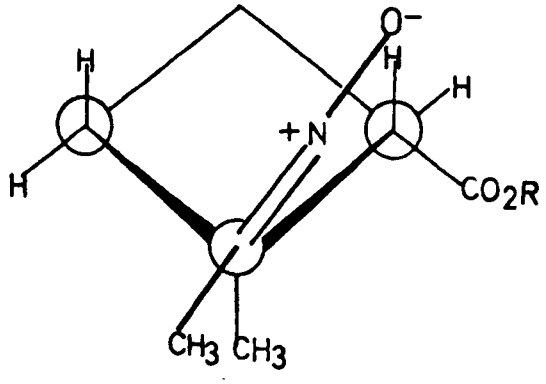

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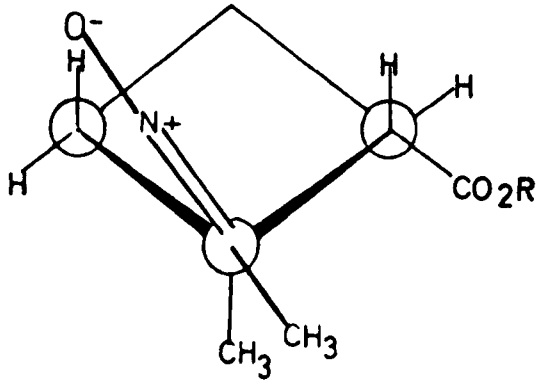


(4)

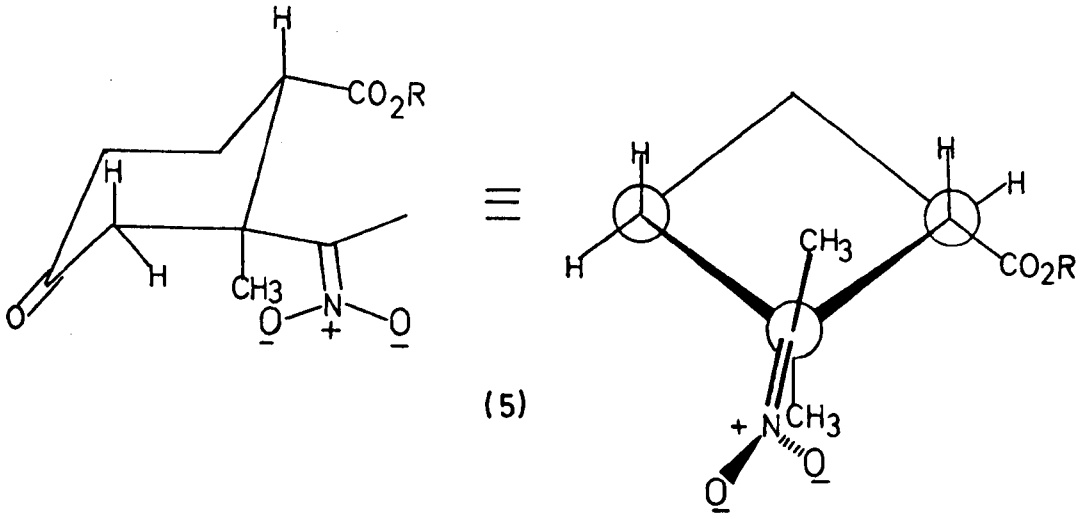
One Nitronate O^- and C=O Group are omitted for clarity

Conformational and Structural Inferences Gained from a Spectroscopic Study of Nitronate Anions

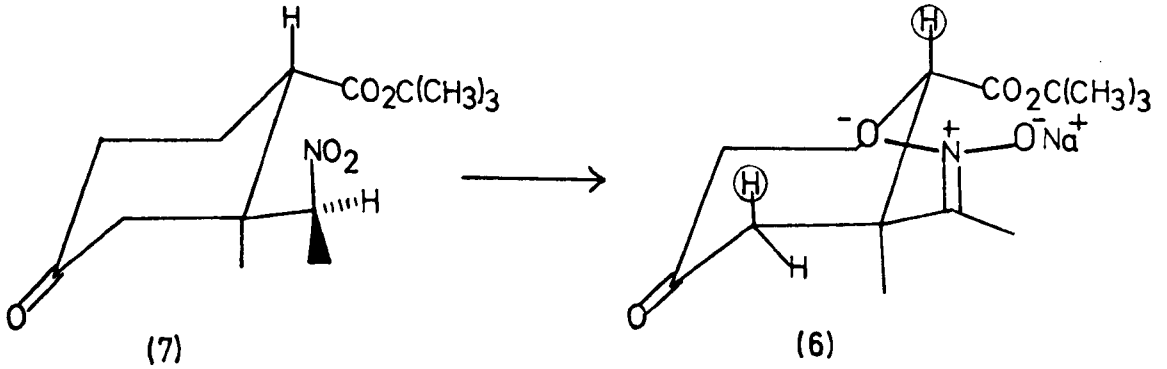
^{1}H Nuclear Magnetic Resonance Studies Involving Ring A Precursors

As indicated previously (Chapter 1, page 21), protonation of the nitronate anion (1), arising from base-catalysed addition[17] of nitroethane to ethyl Hagemann's ester, resulted in a mixture of two isomers : a liquid epimer (2a) and a crystalline epimer (2b). The relative configuration of the nitroethyl and ethoxycarbonyl groupings in the crystalline epimer was unambiguously proved[17] to be trans. Only the trans configuration was of any use for further synthetic work (Chapters 1 and 2). In order to make certain that (2a) and (2b) were isomeric only at the 2-nitroethyl centre, and also related via the same nitronate anion, ^{1}H n.m.r. spectroscopy was used to examine the anions derived from compounds (2a) and (2b). Having established this point, kinetic protonation of the nitronate anion (1) was also studied[17] to determine the stereoselectivity of this process, which was possibly of relevance to synthetic studies with these compounds, involving bromination and carbon-carbon bond formation (Chapters 1 and 2).

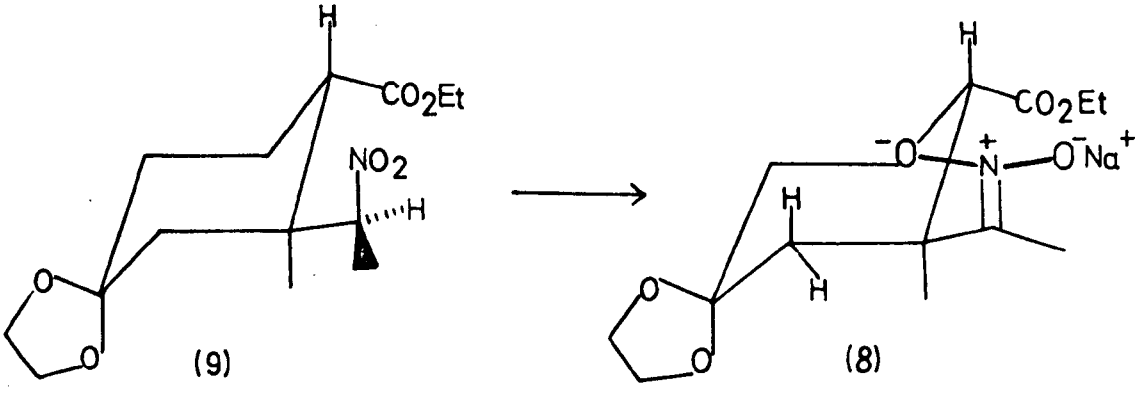
Reacting either epimer (2a) or (2b) with an equivalent of sodium hydride in $^2H_6$-DMSO quantitatively gave the nitronate anion (1). The ^{1}H n.m.r. spectrum of this anion showed interesting and unusual chemical shift differences when compared with the spectrum of the parent nitro compound. In the anion (1) the proton H-4 is shifted to δ 4.46, 1.76 p.p.m. downfield from its position in the parent. Its identity is evident from the approximate quartet nature of the signal; H-4 being the X portion of an ABX system. Coupling constants have not been calculated as the AB portion cannot be clearly defined in the spectrum. Also in (1) a doublet (J = 13 Hz) at δ 4.20 is superimposed on the methylene quartet of the ethyl group. The magnitude of the coupling constant indicates geminal coupling and this resonance is assigned to H-2ax in the conformation of (1) shown. (For an explanation of this assignment, see below). The assignment of H-4 in the anion (1) was confirmed by deprotonating the nitro compound (2b) using sodium $^2H_3$-methoxide in $^2H_4$-methanol. Under these conditions spectral simplification occurs due to complete deuterium exchange at C-2 and C-6, as well as formation of the nitronate anion. Also, H-4 moves from δ 2.88 in the parent nitro compound to δ 4.46 ($J_{AX} + J_{BX}$ = 5 or 16 Hz) (See figures 1 and 2 respectively). The only other protons in (1) experiencing a deshielding effect are


H
CO2R
H
H
CH3
O
N
O
≡
H
H
H
H
CH3
CO2R
+
N
CH3
O
O

(5)


H
CO2C(CH3)3
NO2
H
O
H
CO2C(CH3)3
O
N
O Na+
H
H
O

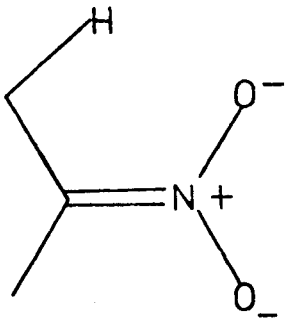
(7) (6)


H
CO2Et
NO2
H
O
O
H
CO2Et
O
N
O Na+
H
H
O
O

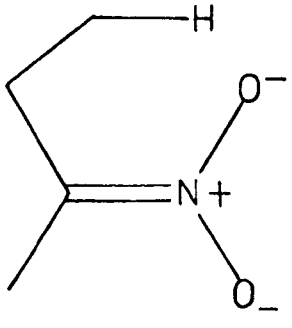
(9) (8)

methyl protons adjacent to the nitronate, but the effect on these is considerably less than that on H-4 due to rotational averaging in the former case. In $^{2}H_{4}$-methanol, $\underline{CH}_3CHNO_2$ in (2b) is at δ 1.52, moving to δ 2.0 on anion formation, and in $^{2}H_{6}$-DMSO the corresponding chemical shifts are δ 1.48 and δ 1.89 respectively (see Table 1 for the complete assignments). The origin of these interesting effects requires an explanation. The difference in free energy (A-value)[112] between an axial and equatorial 2-nitroethyl group is likely to lie between that of a methyl group (1.8 Kcal mole^{-1}) and that of an isopropyl group[113] (2.1), i.e. ca. 2.0 Kcal mole^{-1}. The A-value for an ethoxycarbonyl group[113] is 1.1 Kcal mole^{-1}, so for the trans configuration, both the nitroethyl and ethoxycarbonyl groupings are very likely to be equatorial as shown in (1) and (2a, 2b). In the anion (1), if the conformation depicted by (3) is significantly populated, then H-4ax lies in the plane of the nitronate anion and should be appreciably deshielded, by analogy with such an effect observed for the neutral nitro group in nitro-alkanes[114] and-arenes.[115] This effect would not be expected to be appreciably dependent on solvent, i.e. whether protic or aprotic (assuming similar populations for the conformations) and the experimental observations support this. To account for the other low field resonance observed at δ 4.20 in $^{2}H_{6}$-DMSO, conformer (4) must also be appreciably populated causing deshielding of only one of the protons at C-2. From Dreiding molecular models, it is evident that only H-2ax satisfies the requirement of being in the same plane as the nitronate group when this is co-planar with H-4. Another possible conformation for this compound is that shown in (5) which relieves some vicinal steric interactions, but also incurs eclipsing of the nitronate group with the ring methyl group. This latter interaction should cause strong deshielding of the methyl group. However, there was no significant difference in chemical shift for the tertiary methyl group at C-3 in the anion compared to that in the nitro compound, suggesting that conformer (5) is not significantly populated.

Similar effects to those described above are also seen in the n.m.r. spectrum of the nitronate anion (6) derived from the nitroethane adduct of t-butyl Hagemann's ester[16] (7). In this case downfield shifts of 1.47 p.p.m. and ~1.5 p.p.m. are observed for H-4 and H-2ax respectively on formation of the nitronate anion (see Table 2). This implies that conformations (3) and (4) are approximately equally populated, since the alternative conformer would have the nitronate group equidistant



(10)



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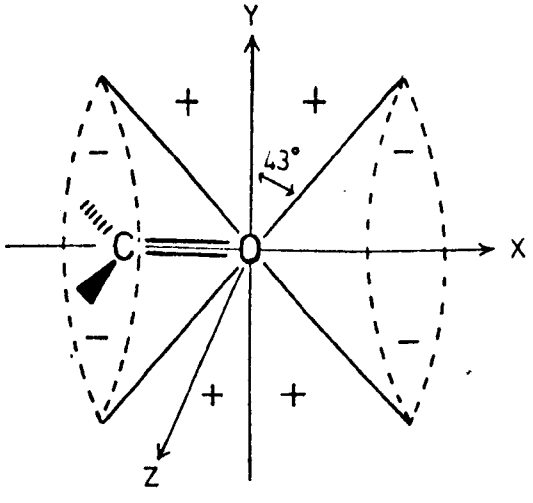
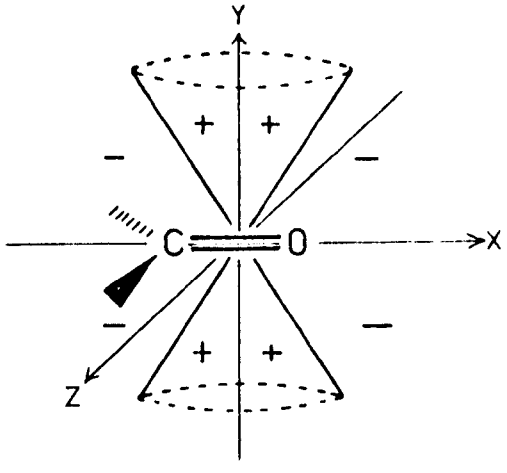


(12)

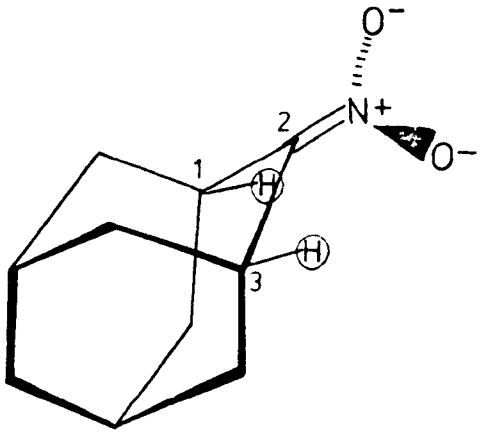


(13)

from H-4 and H-2ax, and this would incur an unfavourable eclipsing of the methyl groups. Further conformational information can be gained from the ethylene ketal derivative of (1), i.e. nitronate (8). On conversion of the parent (9) to the nitronate anion (8), H-4 moves downfield by 2.18 p.p.m. to δ 4.58 from its original position at δ 2.40 in (9). The deshielding of H-2ax however is smaller, being shifted by only ca. 1.5 p.p.m. A more accurate measurement is difficult since it is not possible to define the precise chemical shift of this proton, which lies within a multiplet ranging from δ 1.65 - 2.10 (see Table 3). The fact that H-4 is more deshielded than H-2ax indicates in compound (8) the conformation of type (3) is of a greater population than that of (4). This might be expected on the basis of electronic repulsion between the nitronate oxygen atoms and the oxygen atoms of the ethylene ketal. Thus, conformation (3) minimises this interaction, rather than (5) which, as mentioned above, would cause eclipsing of the nitronate and methyl groups with consequent deshielding of the latter. The chemical shift of this methyl group is unchanged on converting the nitro compound (9) to its nitronate anion (8), as was also noted for other compounds in this series (see Tables 1 and 2), thus confirming that the population of conformer (5) is low or even zero.

Hence, from the above and also from further observations (see below), it has been possible to gain information concerning preferred conformations in mobile systems. The deshielding effect on specific protons caused by the nitronate anion can be quantified by these results. In general, whenever the conformation of a nitronate anion causes a proton or protons to lie in or near the plane defined by the atoms of the nitronate anion (as schematically represented by (10) and (11)), then for the cases examined, this proton(s) experiences a downfield shift of ca. 1.5 - 2.0 p.p.m. relative to the standard unperturbed position.[116]

Huitric proposed[114] that the nitro group causes positive shielding in the 'cones' above and below the NO_2 plane, and negative shielding elsewhere, drawing an analogy with the anisotropic long-range shielding effect of the carbonyl group. In an attempt to quantify the effect of the nitro group, Yamaguchi used[117] an n.m.r. study of o-methylnitrobenzene derivatives to calculate the principal magnetic anisotropies of the NO_2 group. However, quantitative assessment of the deshielding effect is difficult, especially in aliphatic situations, due to the uncertainty of rotamer populations about the C-NO_2 bond, and, as yet, no theoretical estimates have been



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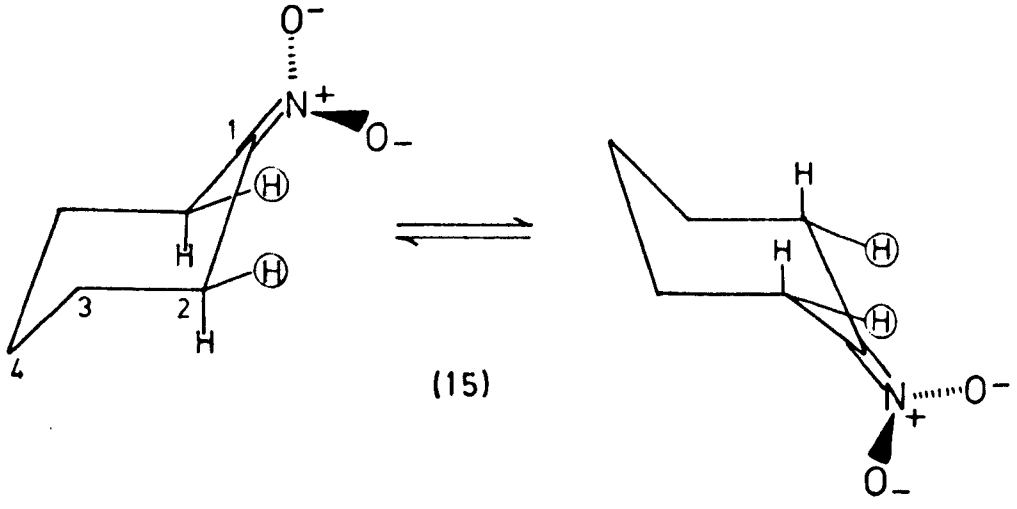

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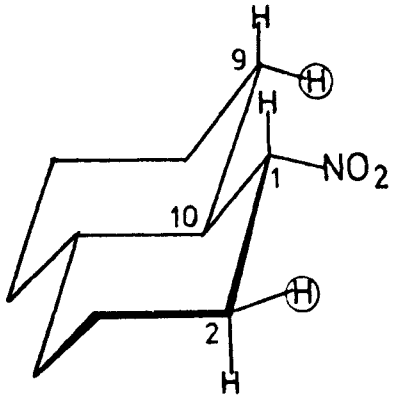
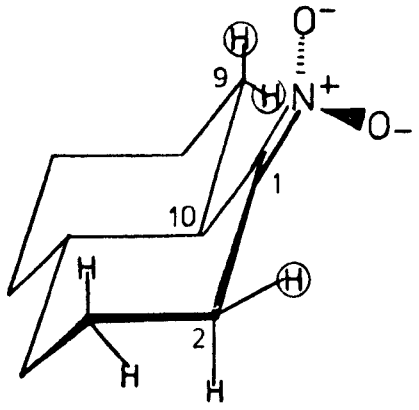


(16)



(17)

reported. The present investigation has not attempted to quantify the diamagnetic anisotropy of the nitronate anion, other than specifying the chemical shift it causes on proximate protons. The nitronate anion should serve as a good model to investigate the magnetic environment around the NO_2 group since the double bond character of the C-N bond does not allow free rotation. ApSimon and his coworkers have used[118] a mathematical approach to redefine the screening 'cone' (12) around the C=O bond, based on data gained from 5 α-androstanes. It is suggested[118] that it cannot be considered a general rule that a proton situated in the plane of a C=O bond is always deshielded. This view is somewhat different from an earlier and widely accepted model (13) proposed by Jackman.[119] Evidence in support of the later findings has appeared.[120] Any attempt to draw an analogy between the carbonyl group and the nitronate group in order to assess the boundaries of positive and negative shielding must be made with some caution in the light of these results.

The interesting findings which arose from studies with the B_{12} ring A precursors encouraged a more detailed study to examine the anisotropy of the nitronate grouping and its effect on protons fixed in a rigid spatial relation to the nitronate anion. It was hoped at the outset that the results might be applied to problems in conformational studies of a general interest. Recently the anisotropic effects of the carboxylate group on the chemical shift of protons have been used[121] to determine conformations in trans- and cis (allo)-hydroxyproline in acidic or basic solution. Roughly, for a specific proton, a high field shift denotes a position above the COO^- plane and a low field shift denotes a location in this plane.[122]

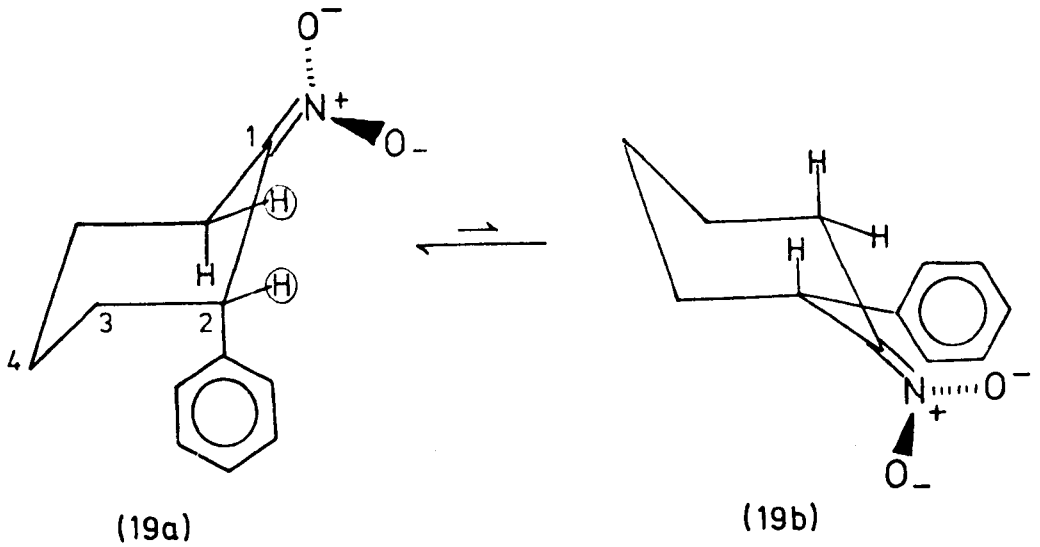
^{1}H n.m.r. Studies in Alicyclic Nitronate Anions

From a comparison of ^{1}H n.m.r. data for 2-adamantyl nitronate (14) (see Table 4) with that for cyclohexyl nitronate (15) (see Table 5 and Figures 3 and 4), some interesting conclusions can be drawn. In the adamantyl nitronate (14) the pseudo-equatorial protons H-1 and H-3 are observed at δ 3.40, whereas for the cyclohexyl nitronate (15) the signals for H-2ax (H-6ax) and H-2eq (H-6eq) are averaged at δ 2.57. It is thus possible to conclude that when the nitronate grouping is exocyclic to a cyclohexanoid ring, as in (14) or (15), then equatorial, but not axial protons at C-2 and C-6, experience a significant downfield shift. In the cyclohexanoid systems examined, the equatorial protons on carbon atoms α to the nitronate grouping resonate at approximately 1.5 p.p.m. downfield from signals for the

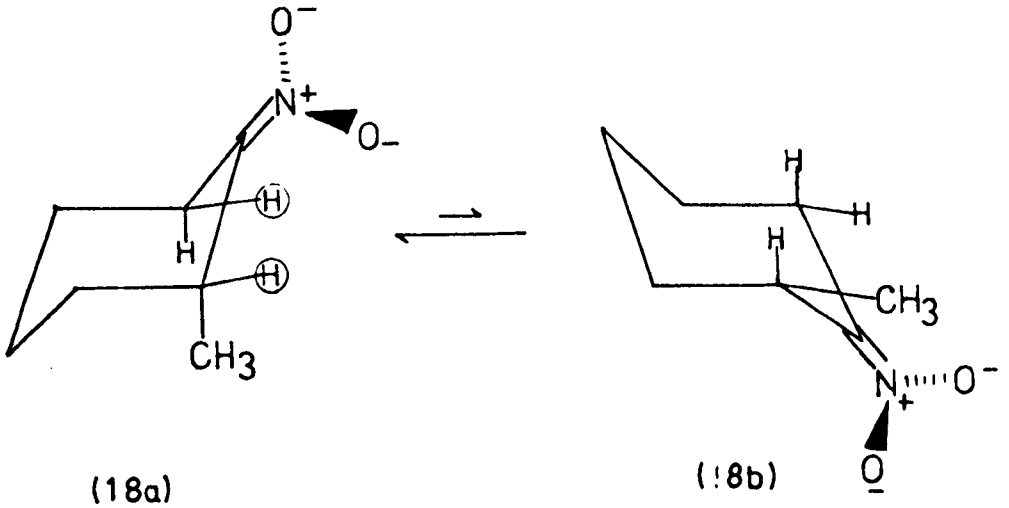
corresponding axial protons.

Trans-trans-1(e)-nitrodecalin (16) is an interesting model for examination since the preferred ring conformation is chair-chair, causing the substituent at C-10 to be equatorial. The rigid ring fusion which exists in the case of trans-decalins, precludes any other chair-chair conformations. The isomer with an equatorial nitro group is the more thermodynamically stable and results from equilibrating a mixture containing trans-cis and trans-trans isomers. Equatorial and axial hydrogens at C-1 are readily distinguished by their differing chemical shifts and coupling constants (see Table 6). When the nitro group is equatorial, deshielding of H-2eq and H-9eq is observed, both signals resonating as a multiplet (δ 1.98 - 2.37) downfield from the remaining proton envelope (δ 0.95 - 1.98). In the nitronate anion (17) however, only H-2eq is significantly deshielded. This assignment is confirmed by the splitting pattern - a doublet of triplets ($J_{H\text{-}2eq\ H\text{-}2ax}$ = 14 Hz, $J_{H\text{-}2eq\ H\text{-}3eq,ax}$ = 5Hz), and its chemical shift (δ 2.73 - 3.05). The splitting pattern indicates that the coupling constants between H-2eq and H-3eq and those between H-2eq and H-3ax are the same. This suggests minimal flattening of the ring, which might have occurred to alleviate the nitronate-alkyl non-bonded interaction. Also, any twisting or interconversion to the chair-boat form, in order to alleviate this interaction, would result in a loss of coplanarity between H-2eq and the nitronate group, which would manifest itself by diminished deshielding of this equatorial proton at C-2. The 'normal' deshielding is observed, however, indicating that the preferred conformation of the nitronate anion (17) is close to the perfect model. Hydrogens H-9eq and H-9ax are both deshielded in the anion, but the chemical shift difference between these two protons is the normal difference observed between equatorial and axial protons. This implies that the plane of the nitronate group bisects the angle between H-9eq and H-9ax, again supporting the argument that there is no twisting of either ring.

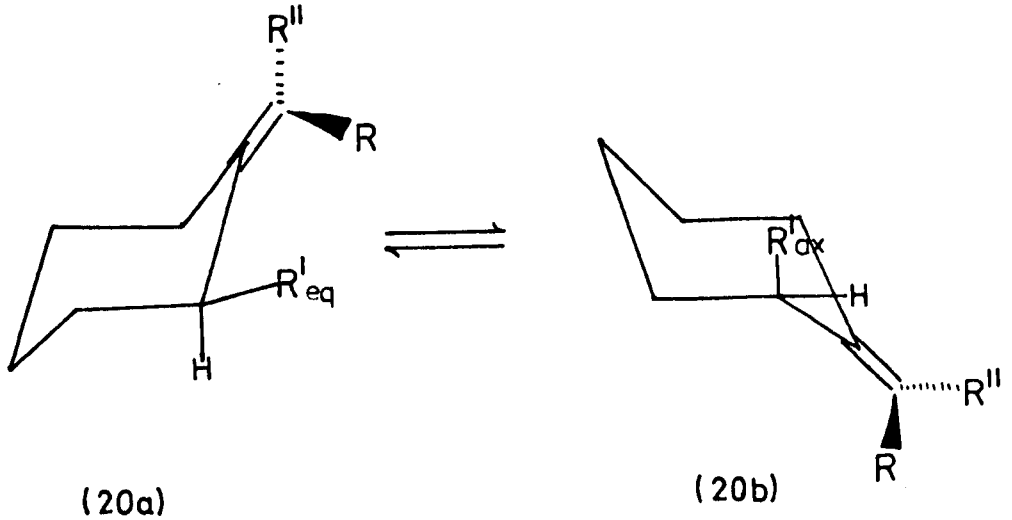
Also in trans-1-nitrodecalin, it was observed that the deprotonation rate of the axial hydrogen at C-1 is very much slower than for the epimer having an equatorial hydrogen at C-1. Since the ^{1}H n.m.r. data suggests minimal flattening of the ring, this implies that the retardation in the rate of deprotonation is caused by a 1,3 interaction with axial hydrogens, a situation which the equatorial hydrogen at C-1 in trans-cis-1-nitrodecalin does not suffer from (see discussion below concerning kinetic protonations and reference 123).



(19a) (19b)



(18a) (18b)



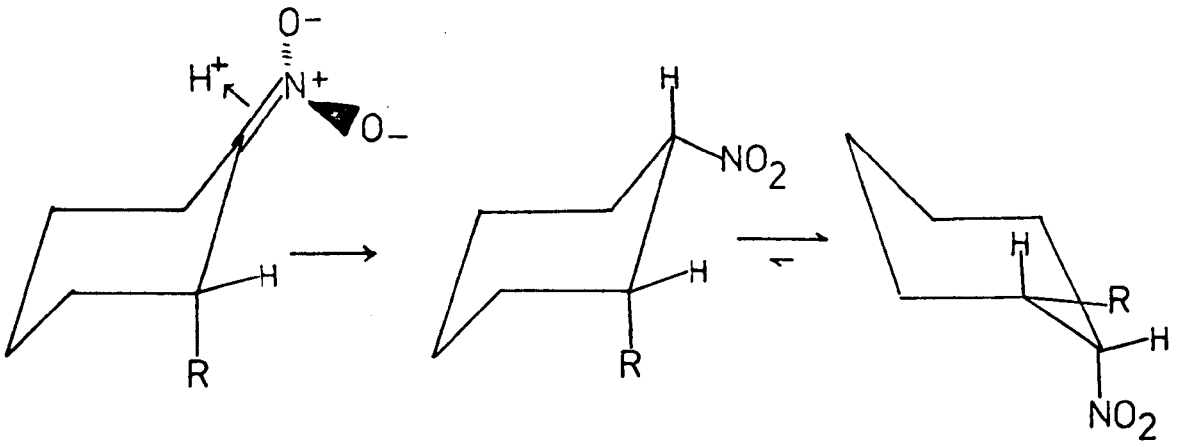
(20a) (20b)

2-Substituted Cyclohexylnitronate Anions

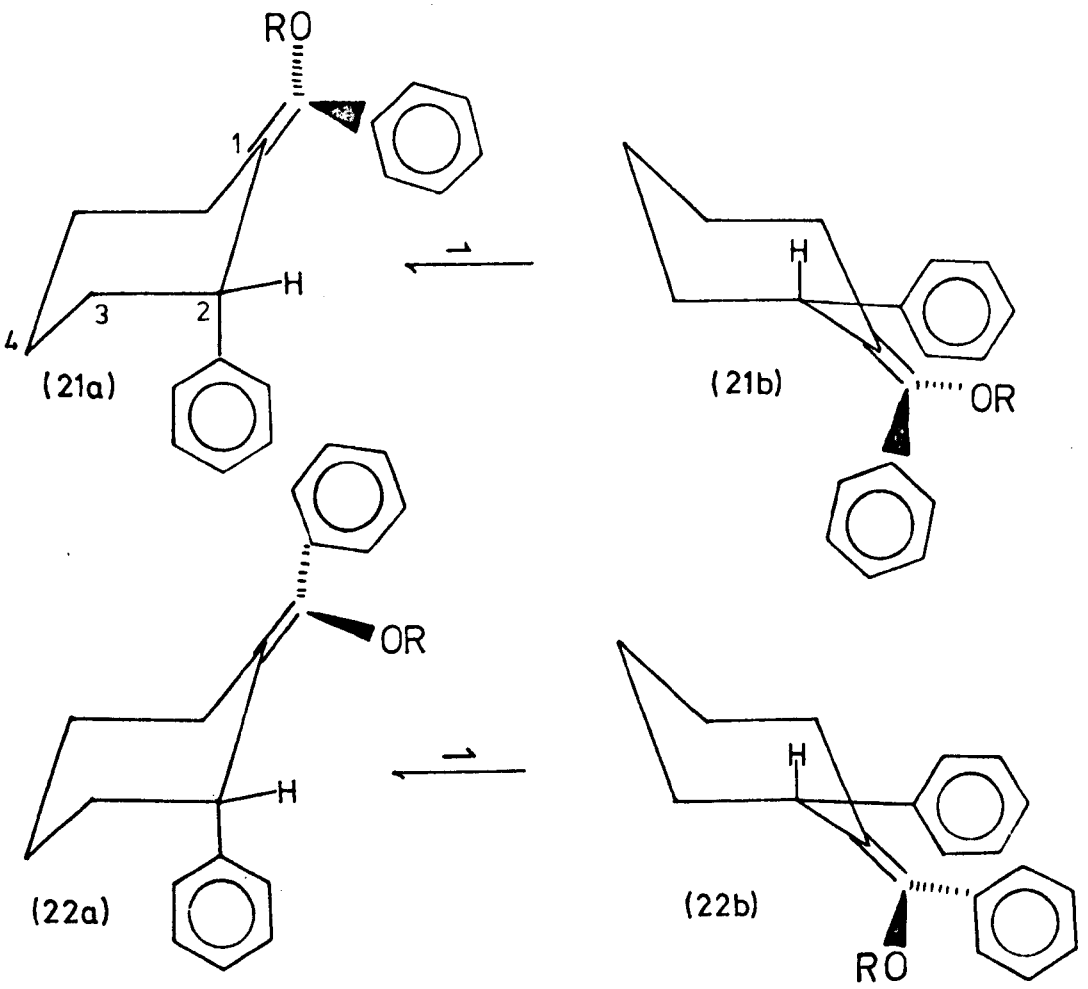
By an application of the deshielding phenomenon of the nitronate anion to more mobile systems, it has been possible to suggest preferred conformations in these systems, especially in 2-substituted cyclohexylnitronates, an area which has been of considerable interest and controversy in past years.

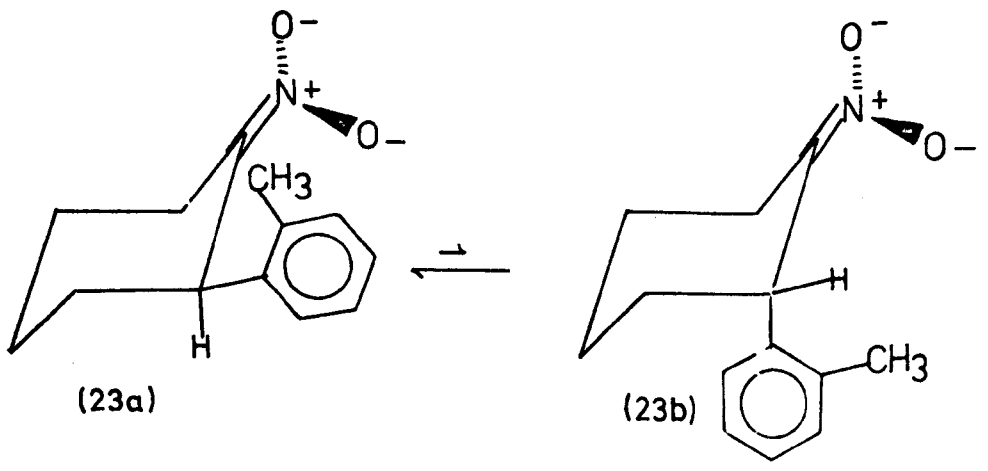
The ^{1}H n.m.r. spectrum of 2-methylcyclohexylnitronate (18) shows two low field multiplets at δ 3.55 and δ 3.12, the higher field signal approximating to a doublet (J = 16 Hz). By comparison with the spectrum of 2-adamantyl nitronate, the signal at δ 3.55 can be assigned to H-2 residing principally in an equatorial position, whilst that at δ 3.12 to the equatorial proton H-6eq. Both signals are well removed from the remaining ring proton multiplet ranging from δ 1.20 - 2.30, and their assignments were confirmed by spin-decoupling experiments (see Table 7). The coupling constants of H-6eq in 2-methylcyclohexylnitronate are different from those of the proton in a similar position in trans-decalylnitronate, i.e. H-2eq (see above). For 2-methylcyclohexylnitronate, H-2eq appears as a doublet (J = 16 Hz) of multiplets ($W_{\frac{1}{2}}$ = 8 Hz and 7.5 Hz), indicating that the coupling constants between H-6eq and H-5eq and those between H-6eq and H-5ax are non-identical. This evidence points to a preferred conformation which may not be a perfect chair. There is opportunity for interconversion of chair conformers in this case, and a preferred conformer with the methyl group in a pseudo-axial position would account for the observed coupling constants of H-6eq.

The ^{1}H n.m.r. spectrum of 2-phenylcyclohexylnitronate (19) (Figure 5) again indicates two equatorial protons in the deshielding plane of the nitronate group. These are assigned to H-6eq at δ 3.20, an approximate doublet, one portion of which is obscured by residual protons in the $^{2}H_{4}$-methanol, and H-2eq as a multiplet ($W_{\frac{1}{2}}$ = 10 Hz) at δ 4.62. In comparison, H-2 in cis-2-phenyl-1-nitrocyclohexane (Figure 6) resonates at δ 3.13 as a sextet. N.m.r. measurements of 2-phenylcyclohexylnitronate at low temperatures showed slight narrowing of the multiplet at δ 4.62 on cooling. A minimum $W_{\frac{1}{2}}$ of 7.5 Hz was observed at 198°K, but further cooling resulted in loss of resolution and line broadening caused by an increase in the viscosity of the solvent (see Table 8). These results and those discussed above strongly suggest that in 2-substituted cyclohexylnitronates the preferred conformation has the 2-substituent in an axial (or pseudo-axial) position as illustrated in conformers



SCHEME I





(18a) and (19a).

The Controversy Surrounding the 'Preferred' Conformation in 2-Substituted Cyclohexanenitronate Anions

Zimmerman demonstrated that kinetic protonation of a number of 2-substituted cyclohexanenitronate[123] anions and cyclohexane enolate[124] anions led to the less stable isomers. For example, protonation of 2-phenylcyclohexylnitronate gave almost exclusively cis-2-phenyl-1-nitrocyclohexane. In this case it was suggested[123] that the less stable isomer arose by C-protonation 'equatorially' from the less hindered side of conformation (19b), which was suggested to be the preferred conformer of the nitronate anion.

Later, Johnson and Malhotra[125] proposed stereochemical theorems to account for steric interference in allylic and pseudo-allylic systems. Empirically it was deduced that molecules which contain an exocyclic or endocyclic double bond would, with the appropriate substitution, exhibit internal non-bonded interactions. These interactions were termed allylic $(A)^{(1,3)}$ and $A^{(1,2)}$ strain respectively. For the case of an exocylic double bond as represented by structures (20a) and (20b), if R and R' are medium or large in size, then the equilibrium should lie in favour of the conformer (20b). If R and R' are small, then the equilibrium should lie to the left, i.e. favouring (20a). Cases where =CR"R represents hetero atoms, for example $=NO_2$, are termed pseudo-allylic. Johnson and Malhotra concluded[125] that for nitronic acids and nitronate anions in the cyclohexane system, their stable conformations will have 2-substituents in axial positions if the substituent is large enough to give rise to a non-bonded interaction with the nitronate group. They anticipated that C-protonation would occur axially from the least hindered side, i.e. trans to the 2-substituent generating largely the cis product (Scheme I).

Zimmerman cast doubt[126] on the validity of the n.m.r. evidence presented by Johnson as a basis for the $A^{(1,3)}$ strain concept. But by comparison of the n.m.r. values of the benzylic proton in (19) with model compounds, in which the orientation of the corresponding hydrogen atom is known, Johnson in a defence[127] refuted this argument completely. Furthermore, an X-ray structure determination[128] for the compounds (21) and (22) showed the 2-phenyl substituent in an axial orientation, and that the alicyclic ring was relatively strain-free. An X-ray analysis of compounds such as 2-phenylcyclohexylnitronate was not attempted due to the instability of

crystalline nitronate salts, but, by analogy with the thermal equilibrium position for 2,6-disubstituted cyclohexanone oximes, it was deduced that for 2-phenylcyclohexylnitronate, conformer (19a) should be preferred.

Bordwell has made several contributions attempting to alleviate this controversy. Initially, from studies on the formation of, and protonation of the nitronate anions from 2-aryl-1-nitrocyclohexanes, he concluded[129] that there was little preference for 'axial' or 'equatorial' approach of the proton donor in the absence of hindering groups at C-2. It was also proposed[129] that in the transition state for protonation of the nitronate anion, the 2-substituent is likely in an axial position, and so exerts a greater steric effect than when in an equatorial position.

In a later, more detailed series of publications,[130] Bordwell offered a new theory. It was suggested that the slight flattening present in cyclohexane rings brings, in the trans-isomer, the 2-phenyl substituent closer to H-1. This proton is therefore shielded from deprotonation, or in the reverse reaction - protonation of the nitronate, it is added more slowly, relative to the unperturbed cis-isomer. The preferred conformation of 2-phenylcyclohexylnitronate anion was also discussed and it was concluded that the 2-substituent was equatorial, the arguments being based partly on a comparison of u.v. data for 2-phenylcyclohexylnitronate and 2-o-methylphenylcyclohexane nitronate (23). The former shows an absorption maximum at 238 nm (ϵ 11,700), whereas in the latter, the addition of the o-methyl group was claimed to cause a dramatic drop in the extinction coefficient, the absorption maximum for (23) being at 237 nm (ϵ 3,000). $A^{(1,3)}$ strain theory predicts that for o-methylphenylcyclohexylnitronate, conformer (23b) should be preferred, but this would not be expected to show anomalous u.v. effects. Bordwell reasons, however, that (23a) is the preferred conformer and that strain is relieved by twisting of the $C=NO_2^-$ group, causing the low extinction coefficient in the u.v. spectrum. By analogy with this result, it is concluded that there is relatively little $A^{(1,3)}$ strain in the 2-phenylcyclohexylnitronate anion and that the preferred conformer has the phenyl substituent in an equatorial position as in (19b).

This is in direct opposition to our conclusions.[116] The information gained from a ^1H n.m.r. study of the 2-phenylcyclohexylnitronate anion, as discussed earlier in this chapter, indicates an equatorial hydrogen at C-2 and the phenyl group in an axial position. These results strongly support the suggestions of Johnson, based on

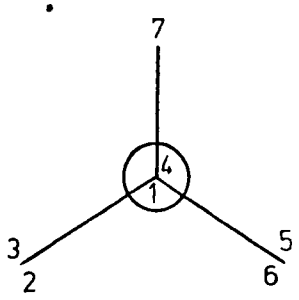
$A^{(1,3)}$ strain and contradict those of Bordwell.

The configuration and conformation of several 2-substituted cyclohexanone oximes and their hydrochlorides have been examined[131] by i.r. and n.m.r. spectroscopy. It was found that for the substituents OEt, Cl, $OCOCH_3$, OCOPh, piperidinyl and morpholinyl, the substituent is axially oriented. For a 2-methyl substituent the equatorial conformation is preferred in the oxime, but in the hydrochloride a preference is shown for the axial conformer. In non-polar solvents a 2-hydroxyl substituent is held in the equatorial position by intramolecular hydrogen bonding between the hydroxyl group and the nitrogen atom of the hydroxyimino group. The hydroxyl group shows an axial preference in polar solvents.

More recently, Russell has applied[132] electron spin resonance to conformational problems by the use of cyclohexanone iminoxy radicals. From an analysis of hyperfine splitting constants, it was concluded that for cis-2,6-dimethylcyclohexanone iminoxy radical the preferred conformation has both alkyl groups in axial positions, indicating that the 2,6-diaxial methyl-methyl interaction is smaller than a syn methyl-oxygen interaction. This phenomenon is also observed in the oxime and offers further support for the theories described concerning pseudo-allylic non-bonded interactions. However, it is noteworthy that the 2- and 4-alkyl and cis-3,5-dimethylcyclohexanone iminoxy radicals prefer the conformation in which the alkyl groups are in an equatorial position.

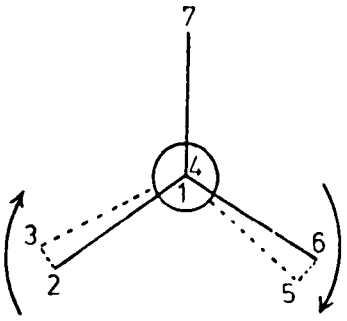
2-Norbornyl and 2-Bornylnitronates

The limited torsional freedom of the norbornane and bornane skeletons make them interesting models for studying deshielding effects caused by the nitro and nitronate groups. One of the main attractions of the norbornane and bornane systems for testing many theories has been their supposed rigidity. It must be noted, however, that X-ray diffraction and valence field-force calculations have shown[133] that these bridged systems can, by undergoing deformation modes called synchro and contra twisting, adapt themselves to strain induced by certain substituents. In general for norbornanes, substituents on C-1 have a very weak effect, if any, giving rise to a contra twist (see projection along C-1 ---- C-4 for (25)). Substituents on C-2, C-3, C-5 and C-6 cause synchro twisting of the norbornane skeleton (see (26)). Bornanes display a weak contra twist, unless substituents are present on C-2 or C-3 which cause more contra twisting. These observations need to be borne



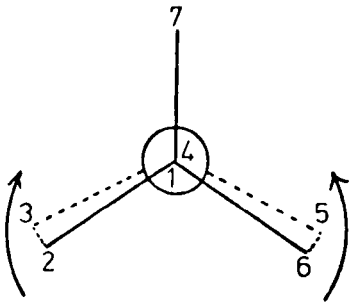
UNDISTORTED

(24)



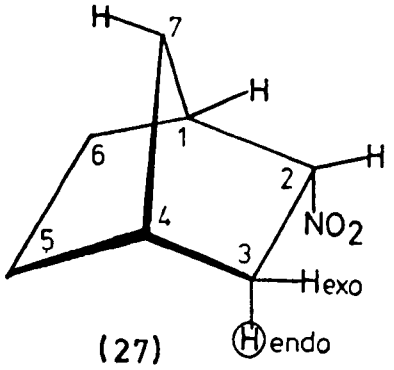
SYNCHRO

(26)

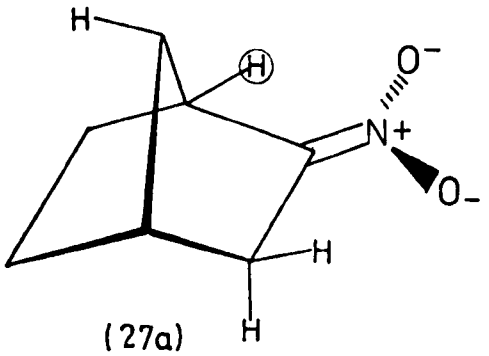


CONTRA

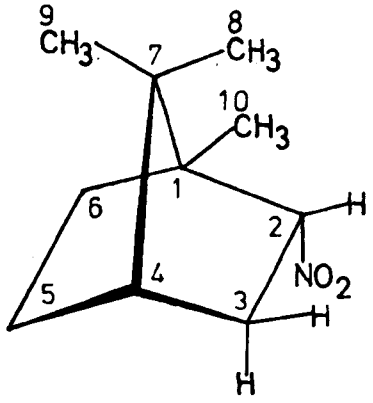
(25)



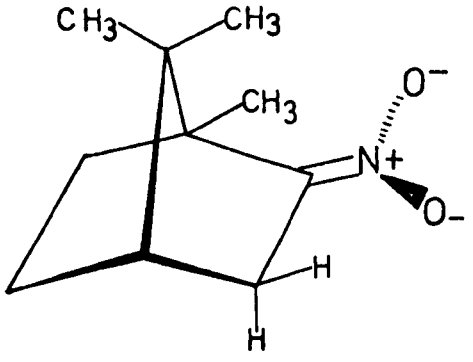
(27)



(27a)



(28)



(28a)

in mind during any conformational inferences made from n.m.r. observations.

2-Nitronorbornane and its Anion

The method of preparing 2-nitronorbornane (27) involved protonation of its anion, and gave a mixture of isomers, but the isomer with an endo nitrogroup predominated, the ratio of endo : exo being ca. 4:1. This mixture was used directly for preparing solutions of the nitronate anion (27a) for n.m.r. studies. A careful analysis of the n.m.r. spectrum of 2-nitronorbornane (27) is necessary before the assignments in the spectrum of its anion (27a) can be made (see Table 9, Figures 7 and 8).

There are several features in the spectrum of 2-nitronorbornane which are worthy of note. Firstly, there is a chemical shift difference between the bridge head protons H-1 and H-4, the chemical shift of each being δ 2.82 and δ 2.34 respectively. This difference is consistent with Huitric's observations[114a] for cis-4-t-butylnitrocyclohexane in which only the equatorial protons at C-2 and C-6 are deshielded by an equatorial nitrogroup. It is also possible to assess the effects of the nitrogroup on the endo and exo protons at C-3 in the norbornane skeleton. In norbornane the endo protons resonate at δ 1.18 and the exo protons at δ 1.49, a difference of 0.31 p.p.m.[134] In nitronorbornane however, the endo proton at C-3 suffers a larger deshielding by the nitrogroup than does the exo proton and both resonances appear under a multiplet ranging from δ 1.80 - 2.20 p.p.m. (centre δ 2.08). The remaining protons resonate under an envelope covering the range δ 1.20 - 1.80 and of integral area representing 6 hydrogens. On formation of the nitronate anion (27a) of 2-nitronorbornane, some interesting changes are apparent. H-1 moves to δ 3.22 and, assuming that H-4 in the nitro compound is in an 'unperturbed' position at δ 2.34(compared to δ 2.2 for norbornane), then the real downfield shift experienced by H-1 is 0.88 p.p.m. Examination of a Dreiding molecular model shows that H-1 is not perfectly in the plane of the nitronate group, and it is probable that the deshielding varies with the dihedral angle. From models of cyclohexanoid nitronate systems, the dihedral angle between the plane of the nitronate group and an equatorial hydrogen at C-2 is almost 0°, but from a model of 2-norbornylnitronate, assuming no twisting about the $C=N^{+}$ bond, the angle between H-1 and the plane of the nitronate is ca. 20°. Of course, this does not take an account of the twisting of the carbon skeleton caused by the nitronate substituent at C-2, as discussed above, so it is difficult to assess the true

conformation of the rings. Also, in the nitronate (27a), H-4 is relatively unperturbed from its position in the nitro compound, moving downfield by only 0.1 p.p.m. to δ 2.44. In a conformation for the nitronate which has a perfect boat conformation for the six-membered ring, the plane of the nitronate group bisects the angle between H-3 exo and H-3 endo, subjecting each to a similar deshielding, which is not the case with the nitrogroup in the parent compound (27). As a consequence, an H-3 endo-exo difference reappears in the nitronate (27a) and is seen as a 0.37 p.p.m. chemical shift difference in the n.m.r. spectrum, the exo proton being at lower field (cf. 0.31 p.p.m. for norbornane[134]). Signals from the protons H-3 are observed as an approximate AB quartet (two doublets (J = 17 Hz) of multiplets) and can be unambiguously assigned (H-3 exo at δ 2.38, H-3 endo at δ 2.01) due to long-range coupling between H-3 endo and H-7. This coupling is absent in the spectrum of 2-bornanenitronate (see below).

These observations suggest that there is only minimal deformation of the carbon skeleton in 2-norbornylnitronate and that there is no rotation about the $C=N^+$ bond of the nitronate group. Without an X-ray structure determination, which would probably be difficult, this contention is not easily verified. The normal chemical shift differences of endo and exo protons in norbornanes in general explain the observed differences for protons at C-3 in the nitronate (27a). The n.m.r. spectra of substituted norbornanes are not first order and so a complete analysis is difficult without computer assistance. A chemical approach resulting in spectral simplification would be that of selective deuteration of 2-norbornanone prior to its conversion to the corresponding nitro compound.

2-Nitrobornane and its Anion

The 2-nitrobornane (28), as prepared, was a single isomer having the nitrogroup in an endo position. For the assignment of signals in the n.m.r. spectrum of 2-bornylnitronate (28a) (Table 10), a comparison with the spectra of 2-nitrobornane and 2-nitronorbornane is useful (Figures 9 and 8).

In the spectrum of 2-nitrobornane, as in the case of 2-nitronorbornane, it was not possible to differentiate between the endo and exo protons at C-3 (both resonate at δ 2.20). This assignment is based on similar arguments used for 2-endo nitronorbornane (see above). The bridge head proton H-4 is assigned to a multiplet at δ 1.80, just overlapping a multiplet (δ 0.90 - 1.86) for the remaining

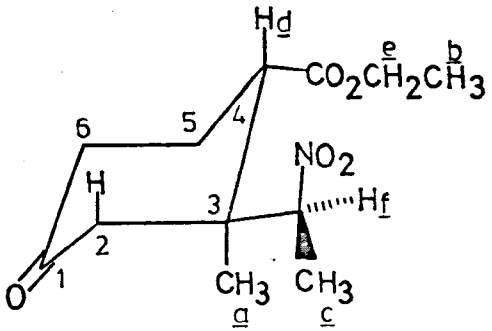
ring hydrogens at C-5 and C-6.

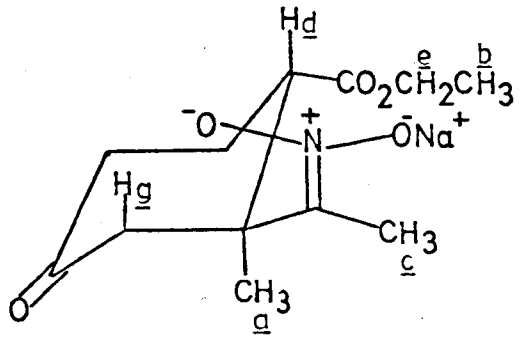
The spectrum of 2-bornylnitronate clearly shows that H-3 endo and H-3 exo are non-equivalent and their assignments are confirmed by the characteristic splitting patterns. On formation of the nitronate anion, H-3 endo moves upfield to δ 2.05 to appear as a sharp doublet showing only geminal coupling (J = 17 Hz). The methyl groups at C-7 effectively prevent long-range 'W' coupling between H-3 endo and a proton at C-7, as was observed in 2-norbornylnitronate. Also the coupling between H-3 endo and H-4 would be expected to be weak or non-existent,[135] strongly supporting this assignment (see Figures 8 and 10 to compare the spectra of 2-norbornylnitronate and 2-bornylnitronate). H-3 exo, however, appears as a doublet of multiplets centred at δ 2.66, exhibiting the expected coupling with H-4, as well as the large geminal coupling (J = 17 Hz) with H-3 endo. Interestingly, H-4 resonates in a multiplet ranging from δ 1.50 - 1.90, a field position much higher than in 2-norbornylnitronate. Also, one, as yet unassigned proton, resides in a high field multiplet (δ 0.90 - 1.30). Formation of the nitronate anion (28a) causes only a small upfield shift (0.08 p.p.m.) on the two bridge head methyl groups at C-7. There is no chemical shift difference between these two methyl groups, indicating that both are outside the positive shielding area of the nitronate group. The methyl group at C-1 suffers a 0.24 p.p.m. downfield shift on anion formation, confirming that it is still in or near the plane of the nitronate group. Consequently, $A^{(1,3)}$ strain undoubtedly exists here, but it must be energetically unfavourable to proceed to a conformation allowing the methyl group to assume a pseudo-axial position. Probably marginal twisting of the envelope conformations of the 5-membered rings occurs, or possibly slight rotation about the $C=N^{+}$ bond of the nitronate occurs to alleviate the $A^{(1,3)}$ strain. This could explain the large chemical shift difference between H-3 endo and H-3 exo (0.59 p.p.m.) observed in 2-bornylnitronate compared to that in 2-norbornylnitronate (0.37 p.p.m.).

Conclusions Regarding the A Strain Theory

The ^{1}H n.m.r. evidence acquired from mobile systems indicates that in situations where $A^{(1,3)}$ strain exists, it can most effectively be relieved by a change to less energetic conformations. In the case of cyclohexanoid systems, this may involve a large group assuming an axial position, rather than suffering an $A^{(1,3)}$ interaction in an equatorial conformation. In rigid systems where there is $A^{(1,3)}$ strain, slight deformation modes occur to alleviate this. If the 'offending' group in a partially mobile system is small, such as the CH_2 group at position 9 in 1-trans-

decalylnitronate, the $A^{(1,3)}$ strain is not great enough to induce a conformational change from a stable conformer (in this case the chair-chair) to one which orients the substituent in a pseudo-axial position.



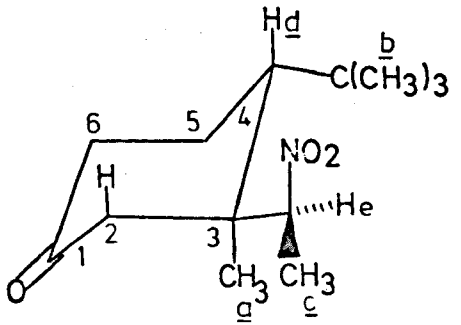


δ (CD_3OD) (100MHz)	δ $(CD_3)_2SO$ (60MHz)	δ (CD_3OD) (100MHz)	δ $(CD_3)_2SO$ (60MHz)
1.16, s, 3H; a	1.08, s, 3H; a	1.16, s, 3H; a	1.07, 3H; a
1.20, t, (J = 7Hz), 3H; b	1.24, t, (J = 7Hz), 3H; b	1.25, s, 3H; b	1.20, 3H; b
1.52, d, (J = 7Hz), 3H; c	1.48, d, (J = 7Hz), 3H; c	1.95 - 2.15, m, 2H	1.90 - 2.50, m, 6H
1.90 - 2.95, m, 7H (1.90 - 2.50, m 2.88, q)	2.00 - 2.50, m, 6H; 2.87, q, 1H; d	2.00, s, 3H; c	1.89, 3H; c
4.20, q, (J = 7Hz), 2H; e	4.19, q, (J = 7Hz), 2H; e	4.46, q, (J = 5 or 16Hz), 1H; d	4.63, q, 1H; d
4.98, q, (J = 7Hz), 1H; f	4.98, q, (J = 7Hz), 1H; f	4.15, q, (J = 7Hz), 2H; d	4.19, q, (J = 7Hz), 2H; e
		H's at C-2, C-6 exchanged in CD_3OD/ $NaOCD_3$	4.20, d, (J = 13Hz), 1H; g

δ H-4 NO_2^- - δ H-4 NO_2 = 1.58 (CD_3OD) ppm
1.76 ($(CD_3)_2SO$) ppm

δ H-2 ax NO_2^- - δ H-2 ax NO_2 = ≃ 1.7 ($(CD_3)_2SO$) ppm

Table 1 4-Ethoxycarbonyl-3-methyl-3-(2'-nitroethyl)cyclohexanone and its nitronate anion



δ $(CD_3)_2SO$ (60MHz)

1.0, s, 3H; a

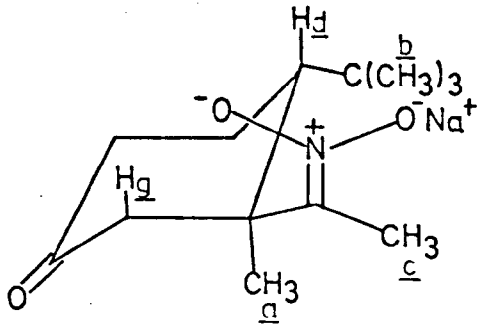
1.46, s, 9H; b

1.57, d, (J = 7Hz), 3H; c

2.00 - 2.50, m, 6H; Ring H's

2.91, m, 1H; d

4.75, q, (J = 7Hz), 1H; e



δ $(CD_3)_2SO$ (60MHz)

1.02, s, 3H; a

1.38, s, 9H; b

1.82, s, 3H; c

1.90 - 2.50, m, 6H; Ring H's

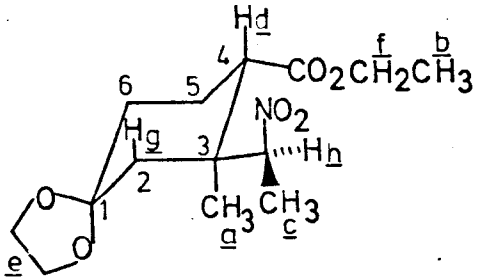
4.38, q, 1H; d

3.98, d, (J = 13Hz), 1H; g

δ H-4 NO_2^- — δ H-4 NO_2 = 1.47 ppm

δ H-2 ax NO_2^- — δ H-2 ax NO_2 ≃ 1.5 ppm

Table 2 4-t-Butoxycarbonyl-3-methyl-3-(2'-nitroethyl)cyclohexanone and its nitronate anion



δ (CD_3OD) (100MHz)

1.25, s, 3H; a

1.25, t, (J = 7Hz), 3H; b

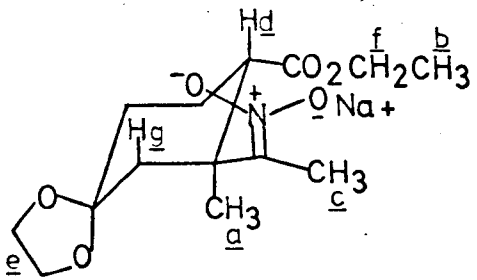
1.65 - 2.1, m, 6H; Ring H's less H-4
(1.50, d, (J = 7Hz), 3H); c

2.40, m, 1H; d

3.96, narrow m, ($W_{\frac{1}{2}}$ = 6Hz); e

4.16, q, (J = 7Hz), 2H; f

4.98, q, (J = 7Hz), 1H; g



δ (CD_3OD) (100MHz)

1.25, s, 3H; a

1.25, t, (J = 7Hz), 3H; b

1.30 - 2.00, m, 5H; Ring H's less H-4 and H-2 ax

2.03, s, 3H; c

3.25, d, (J ≃ 12Hz), partially obscured by solvent, 1H; g

3.96, narrow m, ($W_{\frac{1}{2}}$ = 8Hz), 4H; e

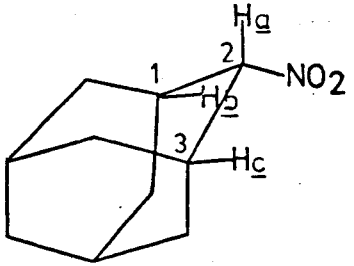
4.10, q, (J = 7Hz), 2H; f

4.58, m, 1H; d

$$\delta \text{ H-4 } NO_2^- - \delta H\text{-}4NO_2 = 2.18 \text{ ppm}$$

$$\delta \text{ H-2 } NO_2^- - \delta H\text{-}4NO_2 \simeq 1.5 \text{ ppm}$$

Table 3 4-Ethoxycarbonyl-3-methyl-3-(2'-nitroethyl)cyclohexanone ethylene ketal and its nitronate anion

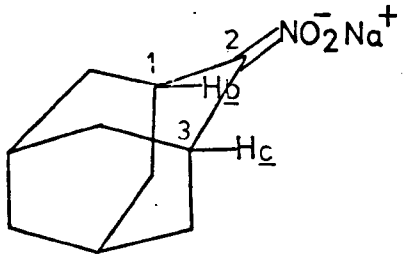


δ (CD$_3$OD) (100MHz)

1.50 - 2.20, m, 10H

2.70, br s, ($W_{\frac{1}{2}}$ = 9Hz), 2H, b, c

4.54, t, (J = 3Hz), 1H; a

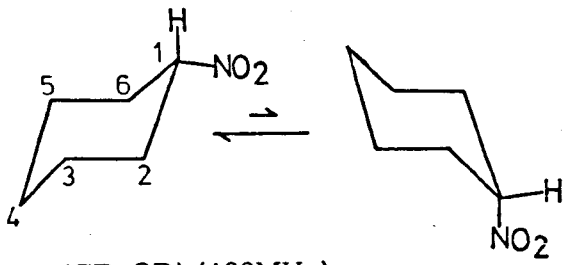


δ (CD$_3$OD) (100MHz)

1.90, br s, ($W_{\frac{1}{2}}$ = 6Hz), 10H

3.40, br s, (partially obscured by solvent), 2H; b, c

Table 4 2-Nitroadamantane and its nitronate anion

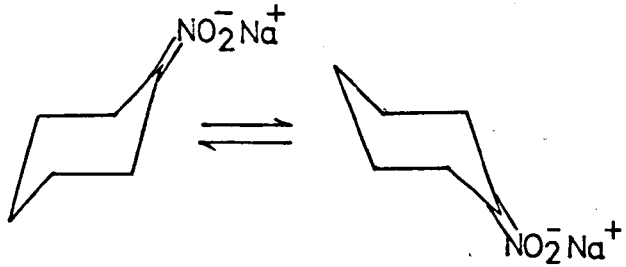


δ (CD_3OD) (100MHz)

1.20 - 2.10, m, 6H; H-3, 4, 5

2.20, m, 4H; H-2, 6

4.64, 9 line m, ($W_{\frac{1}{2}}$ = 24Hz), 1H; H-1

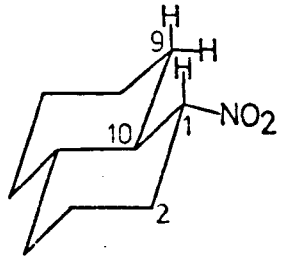


δ (CD_3OD) (100MHz)

1.60, m, 6H; H-3, 4, 5

2.57, m, 4H; H-2, 6

Table 5 Nitrocyclohexane and its nitronate anion

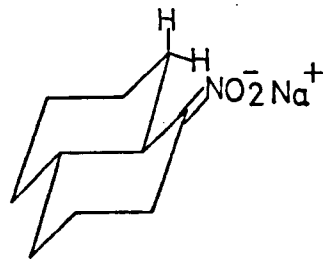


δ (CD_3OD) (90MHz)

0.95 - 1.98, m, 14H

1.98 - 2.37 m, 2H; H-2 eq, H-9 eq.

4.11 - 4.45, m, (sextet J = 13, 4Hz), 1H; H-1 ax



δ (CD_3OD) (90MHz)

0.75 - 1.94, m, 14H

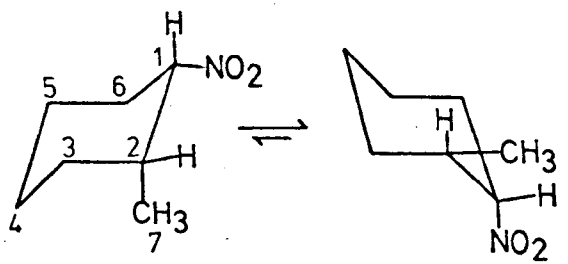
1.94 - 2.73, m, 2H
(1.94 - 2.40, m, 1H; H-9 ax
2.40 - 2.73, d of m, (J = 12Hz), 1H; H-9 eq)

2.73 - 3.05, m, (sextet J = 14, 5Hz), 1H; H-2 eq

N.B. for Trans-cis-1(a)-nitrodecalin(100MHz)

H-1 eq = δ 4.72, m, ($W_{\frac{1}{2}}$ = 8Hz)

Table 6 Trans-trans-1(e)-nitrodecalin and its nitronate anion



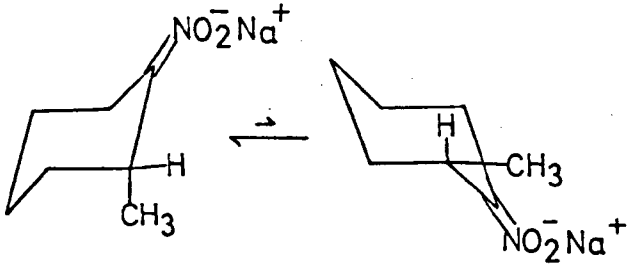
δ (CD_3OD) (100MHz)

0.90, d, (J = 7Hz), 3H; H-7

1.20 - 2.20, m, 8H

2.38, m, 1H; H-2

4.20, q, ($W_{\frac{1}{2}}$ = 13Hz), 0.2H
4.67, sx, (J = 4, 11; $W_{\frac{1}{2}}$ = 22.5Hz), 0.8H) 1H; H-1 cis / H-1 trans



δ (CD_3OD) (100MHz)

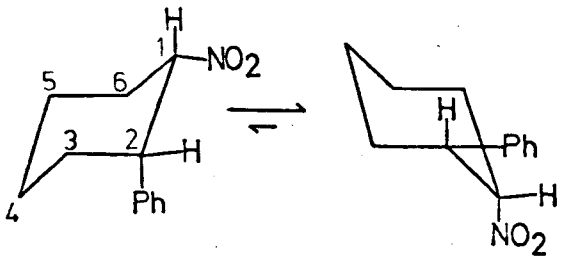
1.10, d, (J = 7Hz), 3H; H-7

1.20 - 2.30, m, 7H

3.12, d of m, (J = 17Hz; $W_{\frac{1}{2}}$ = 8Hz), 7.5Hz), 1H; H-6eq

3.55, m, (partially obscured by solvent), 1H; H-2eq

Table 7 2-Methyl-1-nitrocyclohexane and its nitronate anion



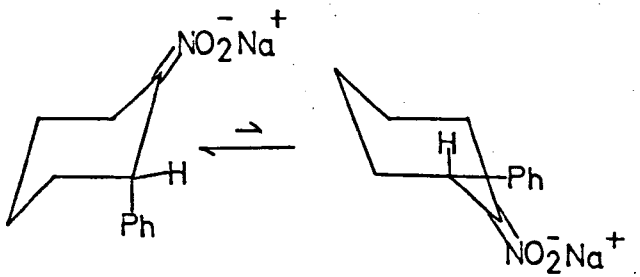
δ (CD_3OD) (100MHz)

1.30 - 2.70, m, 8H

3.13, sx, (J = 12, 4, 4Hz) 1H; H-2

5.02, q, (J = 3, 3, 3Hz) 1H; H-1

7.24, s, 5H; Phenyl



δ (CD_3OD) (100MHz) 298°K

1.20 - 2.30, m, 7H

3.20, d, (partially concealed by solvent), 1H; 6-Heq
4.62, m, ($W_{\frac{1}{2}}$ = 10Hz), 1H; H-2
7.22, narrow m, 5H; Phenyl

δ (CD_3OD) 223°K

1.10 - 2.40, m, 7H

3.20, d, (partially concealed by solvent) 1H; H-6eq

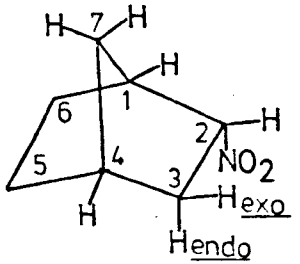
4.58, m, ($W_{\frac{1}{2}}$ = 8Hz), 1H; H-2

7.22, narrow m, 5H; Phenyl

Variable Temperature measurement on H-2; T°K ($W_{\frac{1}{2}}$ Hz)

298°(10), 273(9), 248(9), 243(9), 223(8), 213(8), 198(7.5), 183(9.5)

Table 8 2-Phenyl-1-nitrocyclohexane and its nitronate anion



δ (CD_3OD) (100MHz)

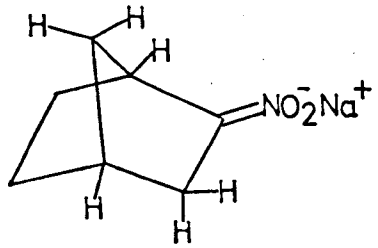
1.20 - 1.80, m, 6H; H's-5, 6, 7

1.80 - 2.20, m, 2H; H-3 exo, endo

2.34, br. s, ($W_{\frac{1}{2}}$ = 8Hz), 1H; H-4

2.82, br. s, ($W_{\frac{1}{2}}$ = 8Hz), 1H; H-1

4.48, q, ($W_{\frac{1}{2}}$ = 12Hz), 0.2H
4.91, quintet, ($W_{\frac{1}{2}}$ = 19Hz), 0.8H) 1H, H-2 endo / H-2 exo



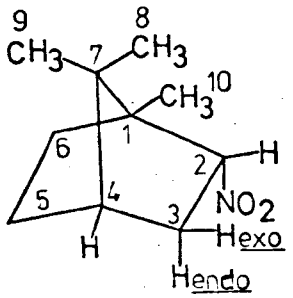
δ (CD_3OD) (100MHz)

1.20 - 1.80, m, 6H; H's-5, 6, 7

1.80 - 2.50, m, 3H
(2.01, d of m, (J = 17Hz, $W_{\frac{1}{2}}$ = 6, 5.5Hz), 1H; H-3 endo
2.38, d of m, (J = 17Hz, $W_{\frac{1}{2}}$ = 6.5Hz, ?), 1H; H-3 exo
2.44, narrow m, ($W_{\frac{1}{2}}$ = 6.5Hz), 1H, H-4)

3.22, narrow m, ($W_{\frac{1}{2}}$ = 5.5Hz), 1H; H-1

Table 9 2-Nitronorbornane and its nitronate anion



δ (CD_3OD) (100MHz)

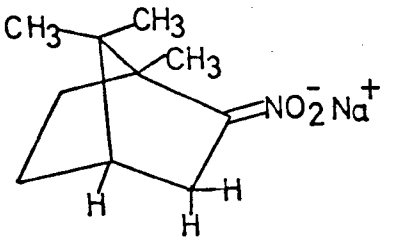
0.96, s, 6H; H's-8, 9

1.08, s, 3H; H-10

0.90 - 1.86, m, 4H; H's-4, 5, 6, 7
(1.80, m, 1H; H-4)

2.20, m, 2H; H-3 endo, exo

4.84, m, ($W_{\frac{1}{2}}$ = 17Hz) 1H; H-2 exo



δ (CD_3OD) (100MHz)

0.88, s, 6H; H's-8, 9

1.32, s, 3H; H-10

0.90 - 1.30, m, 1H; ?

1.50 - 1.90, m, 4H; H's-4, 5, 6 less 1H

2.05, d, (J = 17Hz), 1H; H-3 endo

2.66, d of m, (J = 17Hz, $W_{\frac{1}{2}}$ = 9Hz); H-3 exo

Table 10 2-Nitrobornane and its nitronate anion

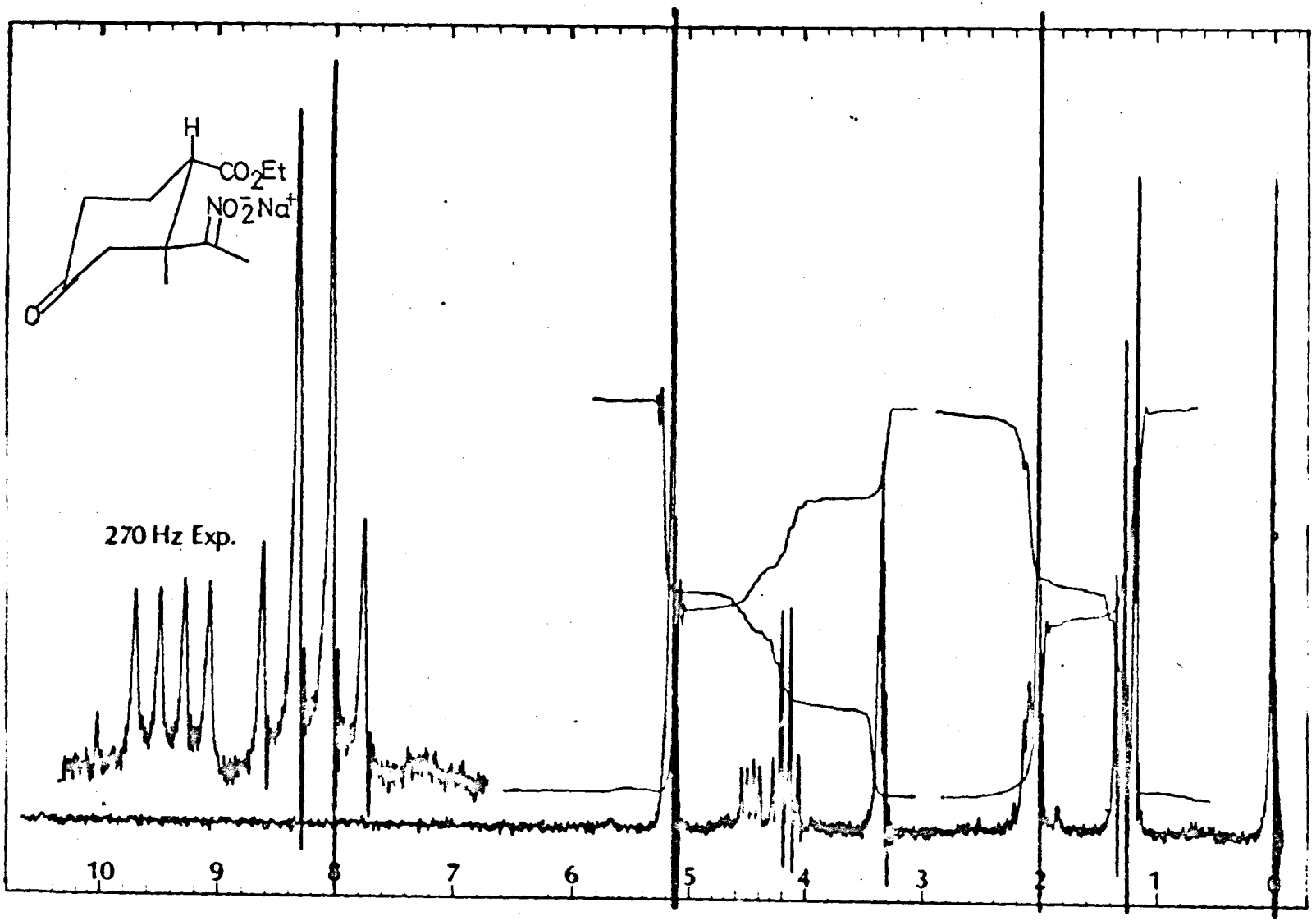


Figure 1 100 MHz(CD3OD) ^{1}H nmr spectrum of 4-Ethoxycarbonyl-3-methyl-3-(2'-ethylnitronate) cyclohexane

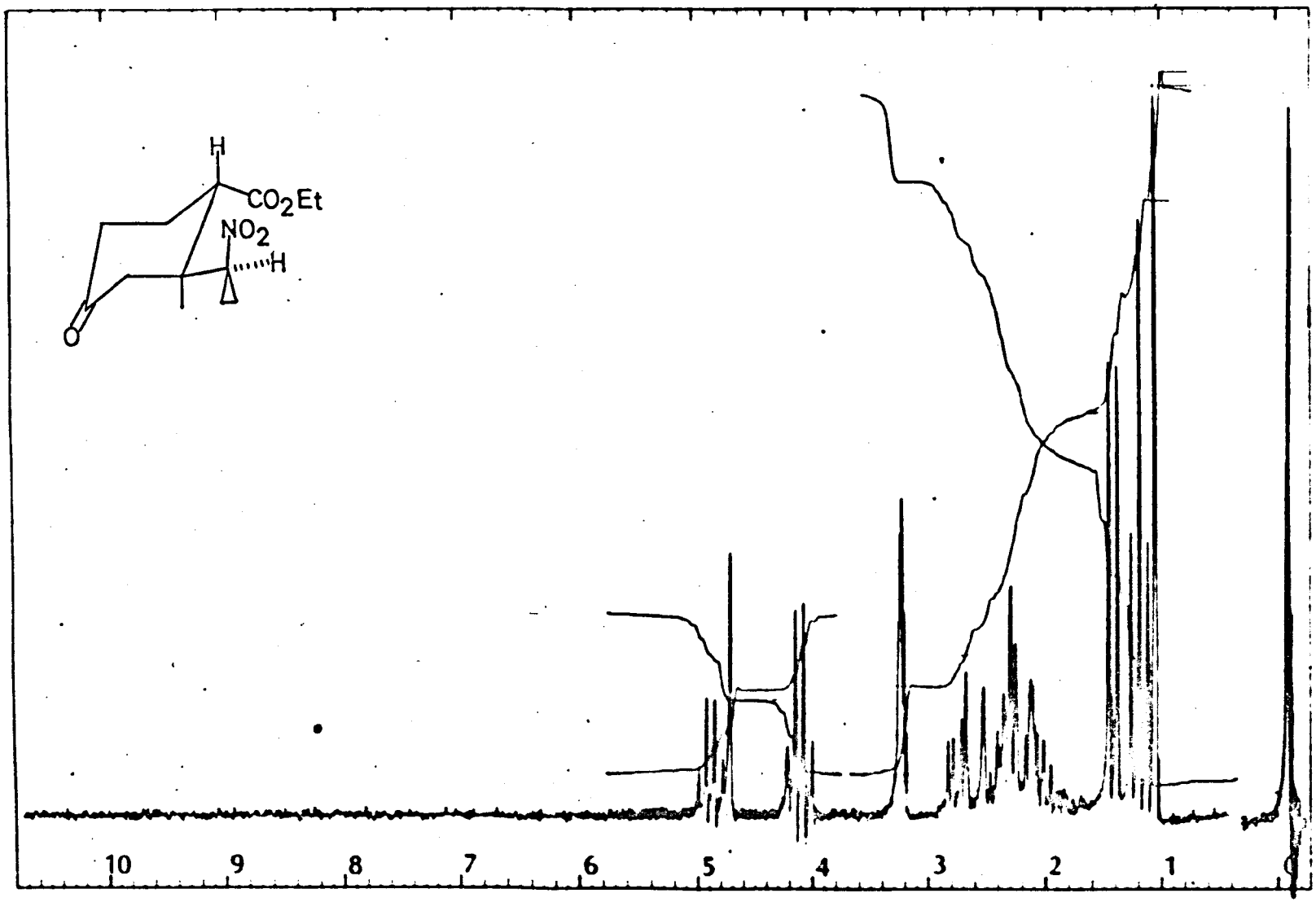


Figure 2 100 MHz(CD_3OD) ^1H nmr spectrum of 4-Ethoxycarbonyl-3-methyl-3-(2'-nitroethyl) cyclohexane

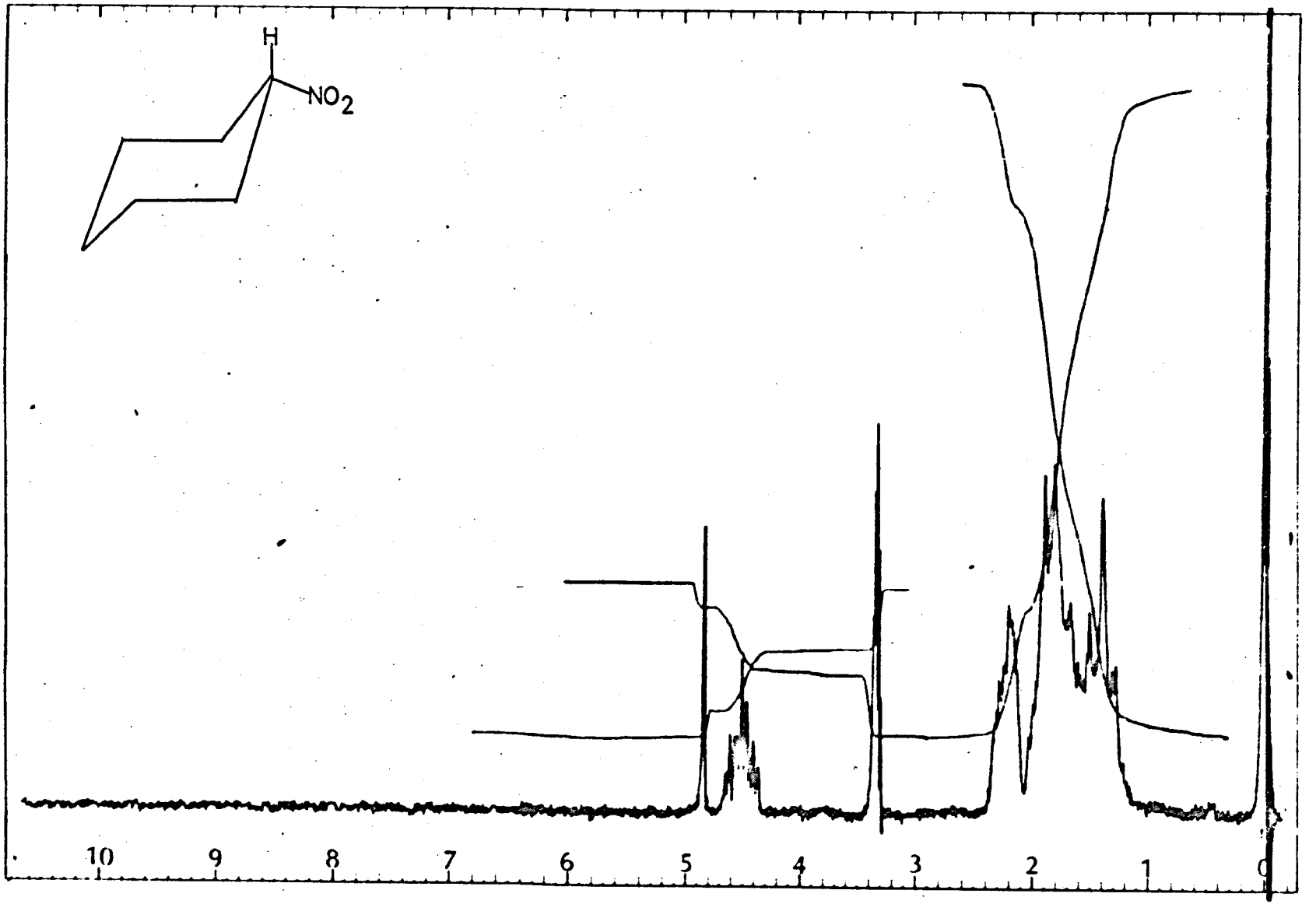


Figure 3 100 MHz(CD3OD) ^{1}H nmr spectrum of Nitrocyclohexane

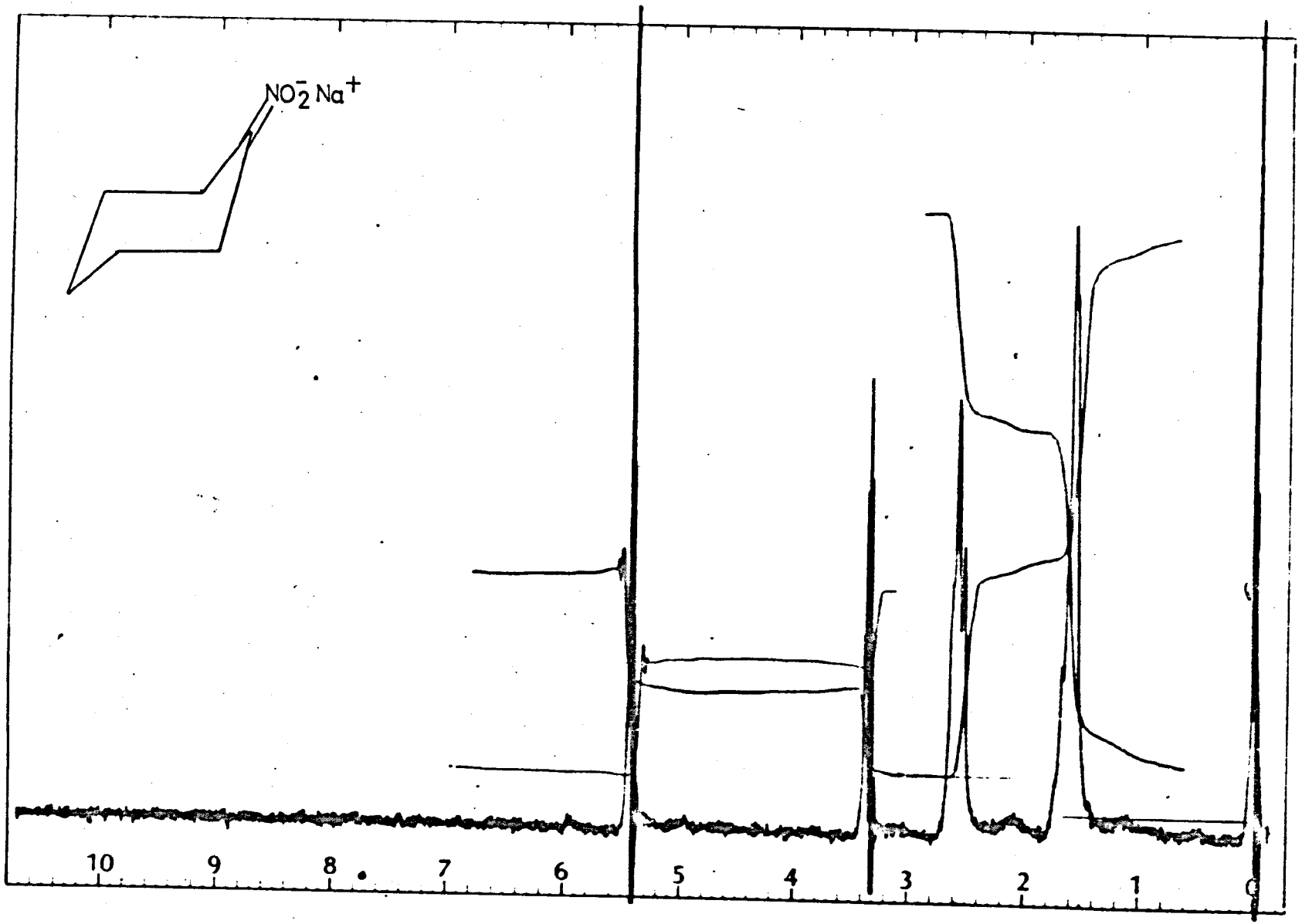


Figure 4 100 MHz(CD3OD) ^{1}H nmr spectrum of cyclohexyl nitronate

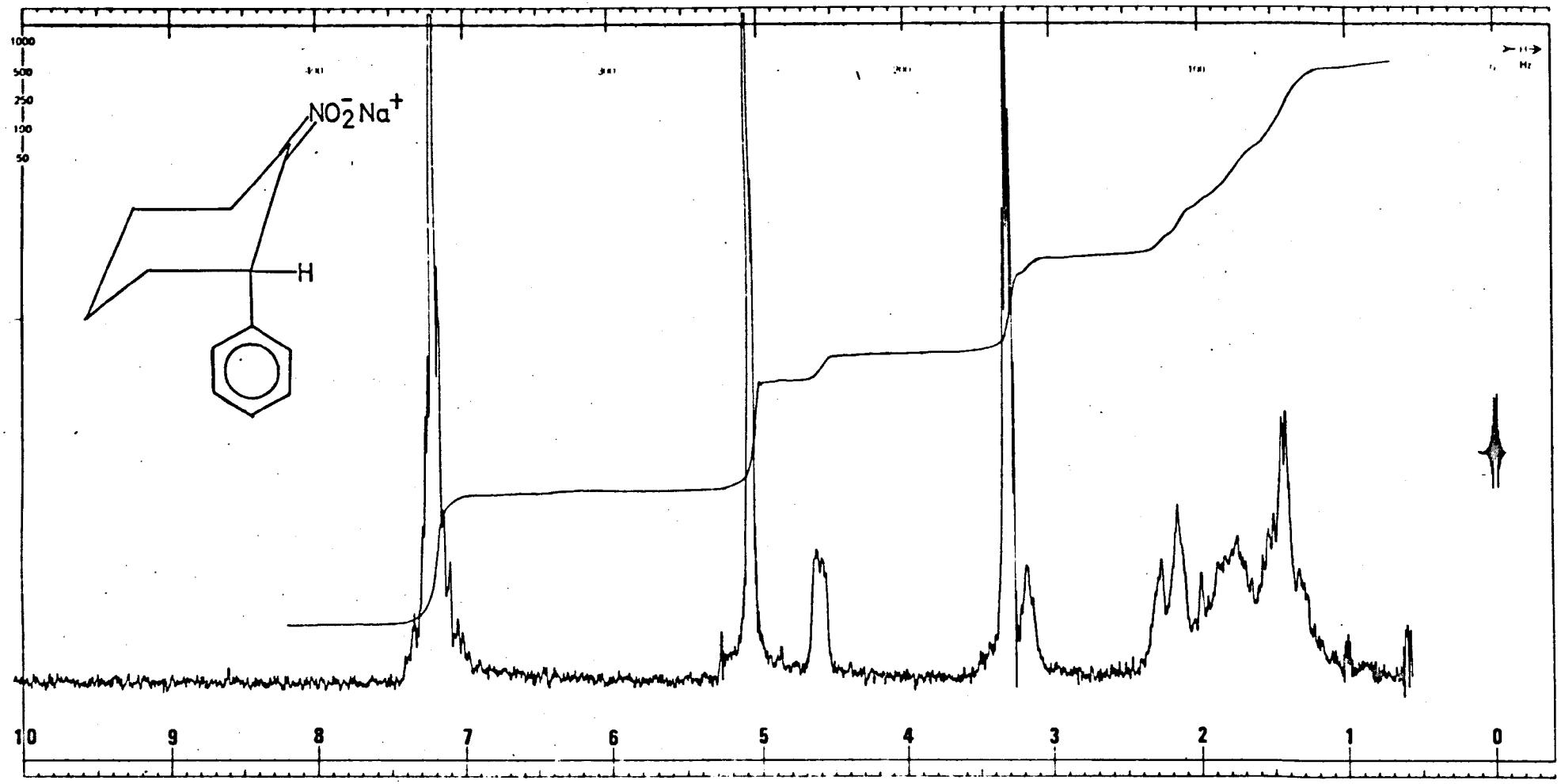


Figure 5 100 MHz(CD_3OD) ^1H nmr spectrum of 2-Phenyl-1-cyclohexylnitronate

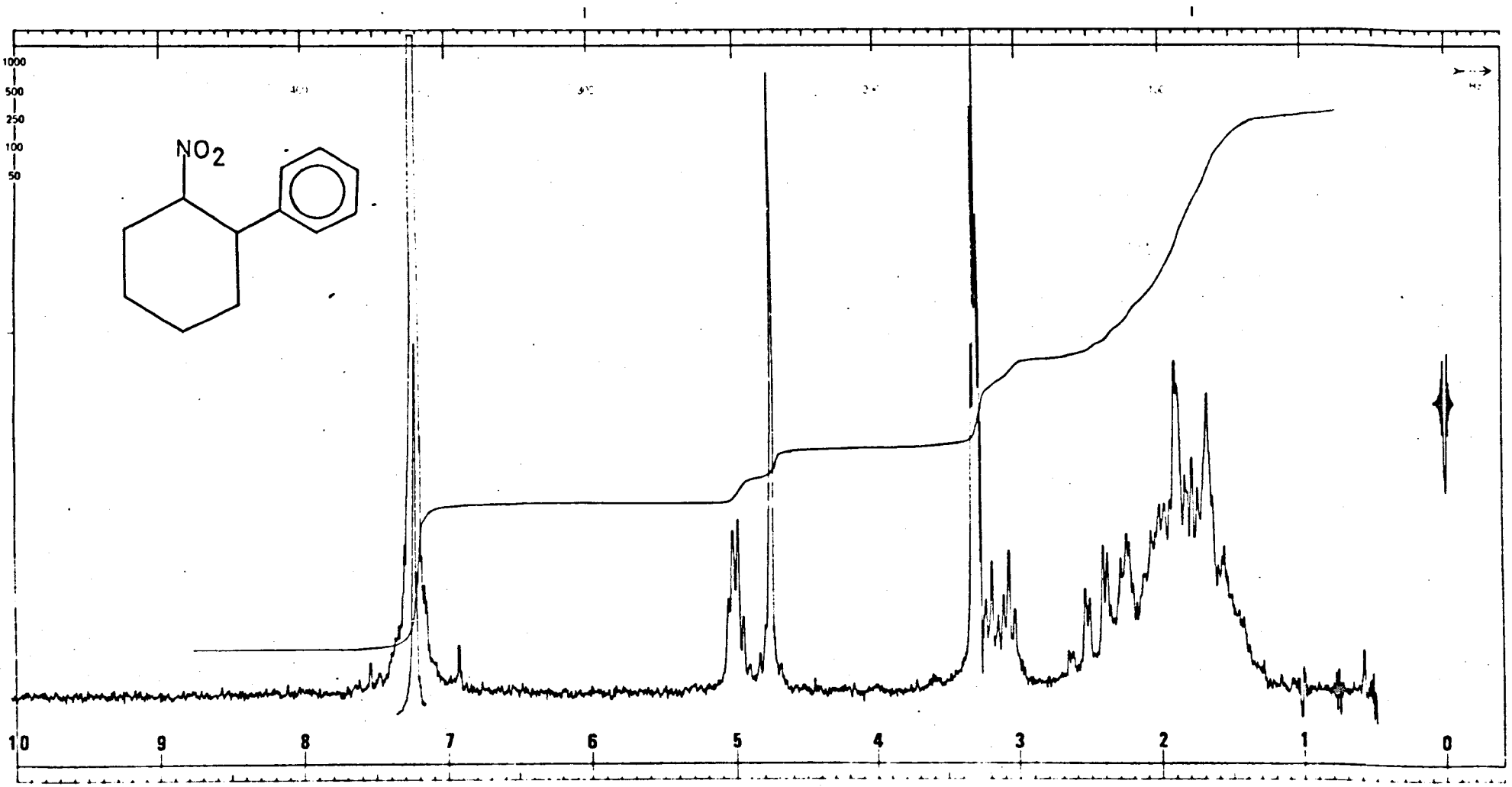


Figure 6 100 MHz(CD3OD) ^{1}H nmr spectrum of cis-2-Phenyl-1-nitrocyclohexane

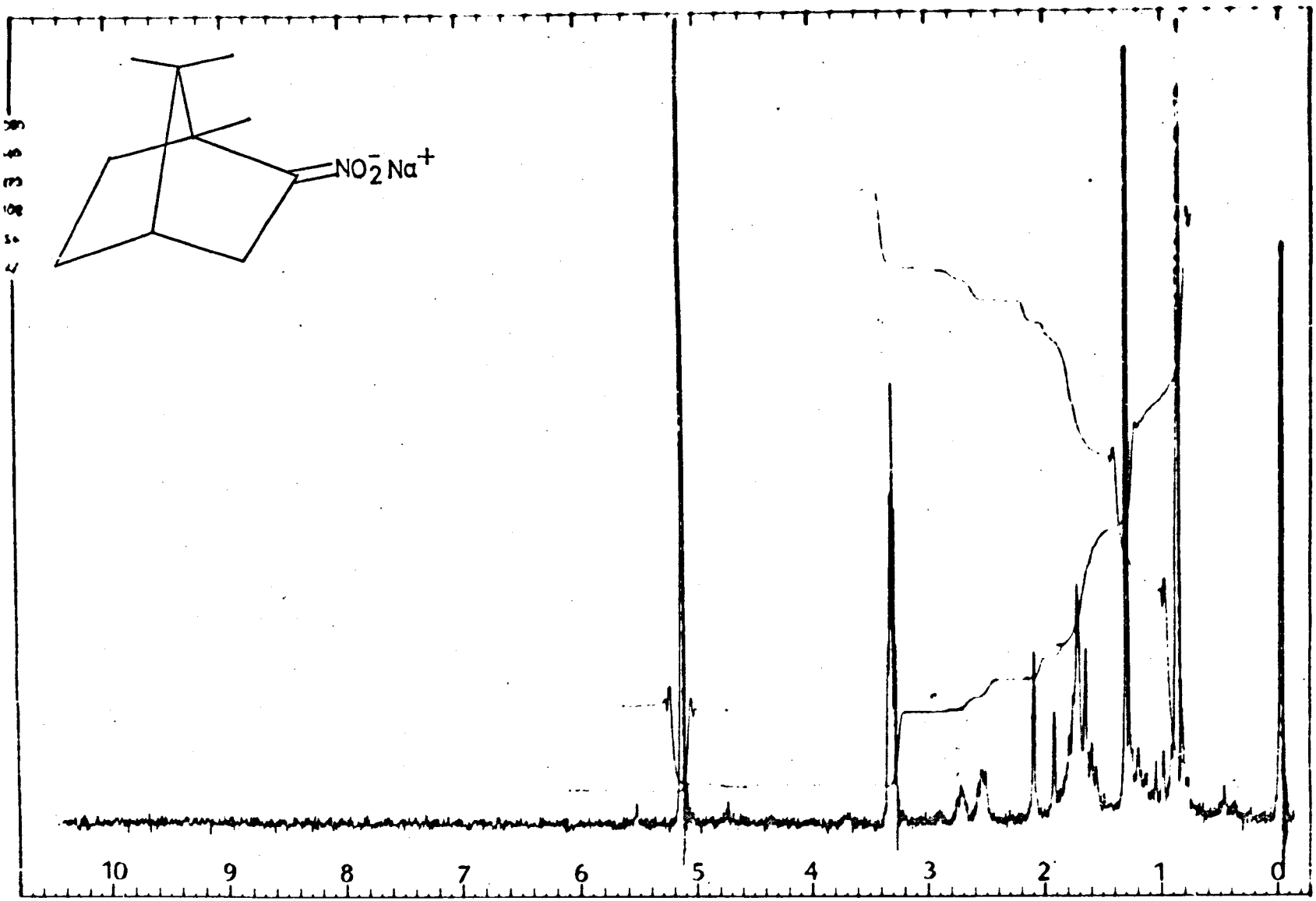


Figure 7 100 MHz(CD3OD) ^{1}H nmr spectrum of 2-Norbornylnitronate

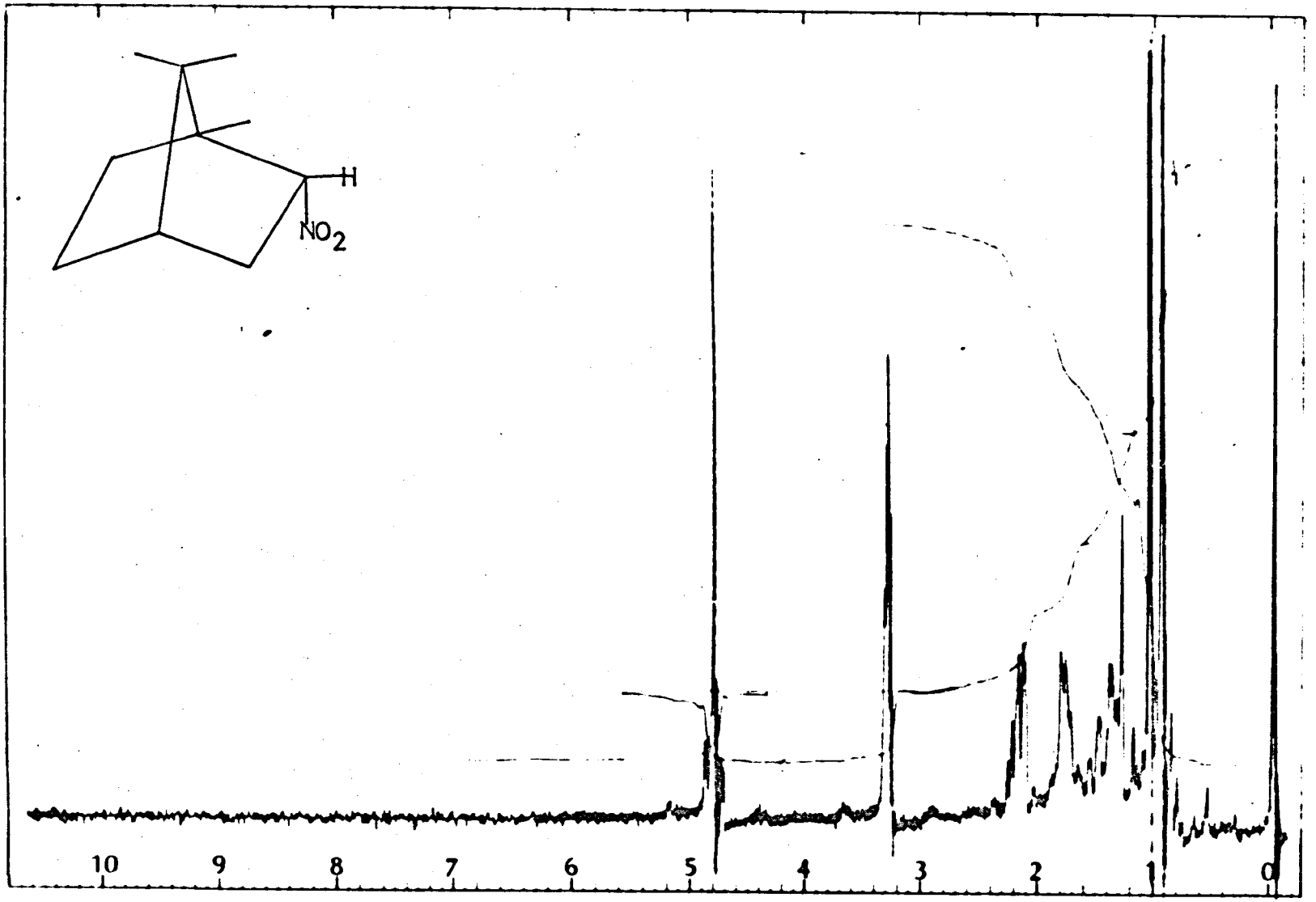


Figure 8 100 MHz(CD_3OD) ^1H nmr spectrum of 2-Nitronorbornane

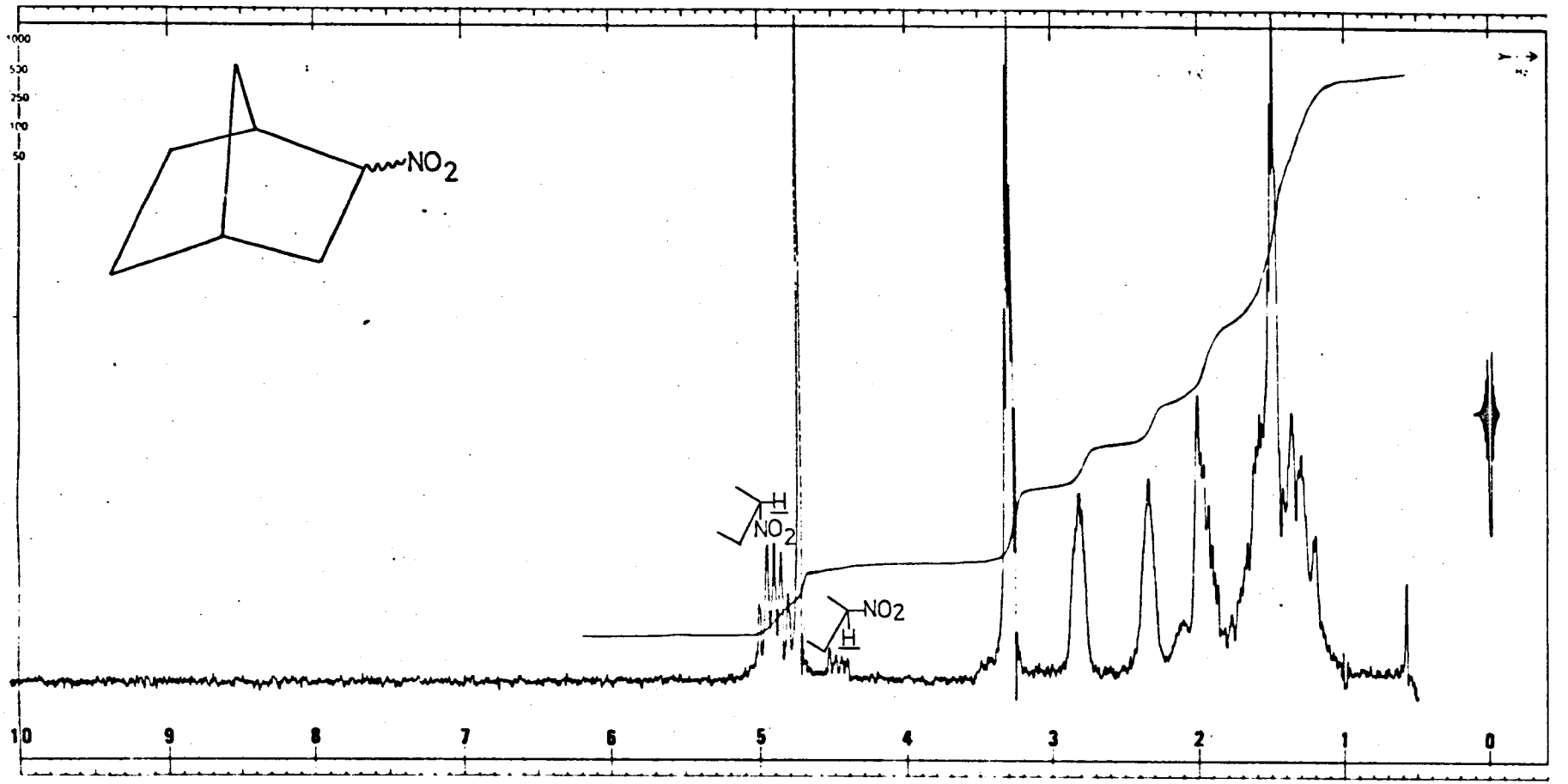


Figure 9 100 MHz(CD3OD) ^{1}H nmr spectrum of 2-Nitrobornane

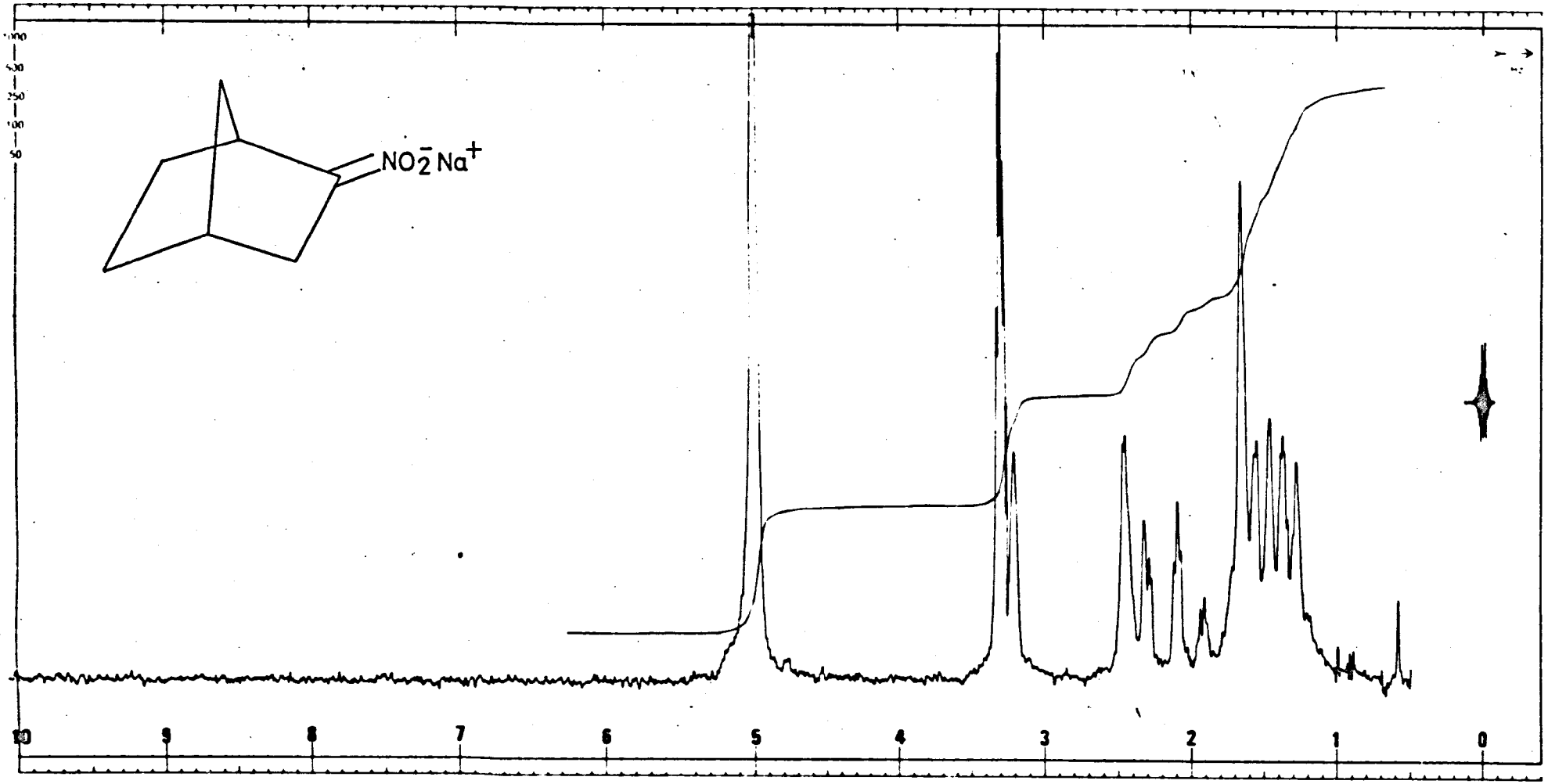


Figure 10 100 MHz(CD3OD) ^{1}H nmr spectrum of 2-Bornylnitronate

^{13}C Nuclear Magnetic Resonance Studies in Nitronate Anions

To date there have been no reports concerning the long-range deshielding effect of the nitro and nitronate groupings on carbon atoms. Since compounds having the appropriate stereochemistry for studying these effects were available, their proton decoupled ^{13}C n.m.r. spectra were examined.

In general, it is observed that large downfield shifts of the order 30 - 45 p.p.m. occur for the nitro-carbon after anion formation, agreeing with results reported for several alkane nitronates.[136] The chemical shift difference between β-carbons in nitro compounds and in their corresponding nitronate anion is relatively much smaller (ca. 3 - 5 p.p.m.). A deshielding of a similar magnitude is also observed for the γ-carbon, depending on the substitution. It has been observed[137] in nitroalkanes that the nitrogroup has a very strong deshielding effect on the α-carbon, but the β-effect is relatively small and decreases progressively with increased methyl substitution on the α-carbon atom. Methyl substitution at the α-carbon causes a slightly diminished α-effect, but the change is proportionately much less (see Table 11).

Table 11

^{13}C Shieldings of Nitroalkanes RNO_2 from TMS[138]

R	α - C	β - C
Me	57.3 (+ 59.4)	
Et	70.4 (+ 64.5)	10.6 (+ 4.7)
i-Pr	79.1 (+ 63.0)	19.1 (+ 3.5)
t-Bu	85.2 (+ 60.0)	26.9 (+ 2.6)

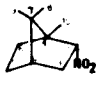
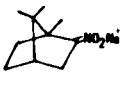
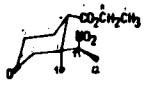
The differences in shielding between the nitroalkane and the parent hydrocarbons are given in brackets.

These trends also appear to be consistent for nitronates, although an increased β-effect is observed (compared to nitro) which may be inductive or may result from an increased interaction between the β-hydrogens and the nitronate oxygens (see Table 12 and note changes caused by solvent).

Solvent Dependence of ^{13}C Shieldings in Nitronate Anions

A significant observation made during this study of nitronate anions was the variation in the chemical shift of the carbon atom of the nitronate group in various solvents. In general, this carbon atom resonated at about 10 p.p.m. to lower field in methanol, compared to DMSO. For example, in the nitronate anion (1), derived

TABLE 12 ^{13}C Shieldings for Nitroalkanes and Nitronate Anions downfield from TMS ppm

COMPOUND	SOLVENT	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	C-12	$C_{NO_2^-} - C_{NO_2}$	$C_{NO_2^-(CD_3OD)} - C_{NO_2(CD_3)_2SO}$
CH_3NO_2	CH_3OH/CD_3OD	63.0													
$CH_2NO_2Na^+$	CH_3OH/CD_3OD	109.8												46.8	
	CH_3OH/CD_3OD	71.4	12.5												
	CH_3OH/CD_3OD	112.3	13.1												
	CD_3OD	78.1	22.0	11.1											
	$(CD_3)_2SO$	77.0	20.3	10.4											
	CD_3OD	119.1	21.6	11.2										41.0	9.8
	$(CD_3)_2SO$	109.3	20.1	11.6										32.3	
	CD_3OD	79.9	21.1	21.1											
	$(CD_3)_2SO$	78.5	20.3	20.3											
	CD_3OD	121.5	19.0	19.0										41.6	9.9
	$(CD_3)_2SO$	111.6	18.2	18.2										33.1	
	CD_3OD	120.9	19.0	19.0										41.0	13.9
	$(CD_3)_2SO$	107.0	18.7	18.7										28.5	
	CD_3OD	[illegible]	93.9	33.4	45.8	28.0	29.7	53.2	200	192	144				
	$(CD_3)_2SO$	49.7	92.3	32.0	43.9	26.9	28.2	51.9	19.4	18.6	13.8				
	CD_3OD	[illegible]	135.1	38.4	45.3	28.4	33.5	52.9	206	193	135			41.2	13.4
	$(CD_3)_2SO$	48.6	121.7	37.7	43.7	27.6	33.2	49.9	20.1	18.9	13.5			29.4	
	CD_3OD	33.1	89.4	32.5	37.8	28.2	38.0	28.2	37.8	37.8	37.8			44.7	
	$(CD_3)_2SO$	31.7	87.6	30.6	36.0	26.1	36.3	26.1	36.0	36.0	36.0				
	CD_3OD	33.3	134.1	33.3	38.3	29.1	37.6	29.1	38.3	38.3	38.3				
	$(CD_3)_2SO$	30.8	[illegible]	30.8	37.2	27.7	36.8	27.7	37.2	37.2	37.2				
	$CDCl_3$	207.9	39.8	42.5	45.9	25.4	46.9	172.4	61.0	13.9	13.9	88.2	17.6		
	CD_3OD	210.5	40.3	43.6	47.2	26.3	48.2	174.2	62.0	14.4	14.4	90.1	18.0		
	$(CD_3)_2SO$	208.5	38.4	42.7	45.5	25.4	47.5	173.0	60.9	142/144	14.4/142	89.1	17.7		
	CD_3OD 1Eq base	216.6	43.6	44.6		24.9		175.6	61.4	14.6	16.2	125.4	22.2	34.3	
	2Eq base	216.8	43.9	44.5	48.1	24.8	50.0	175.6	61.5	14.6	16.1	124.9	22.4		9.9
	$(CD_3)_2SO$	210.1	38.6	43.0	41.5	23.8	48.8	173.9	59.4	14.0	15.6	115.5	21.2	26.4	

from the ring A precursor (2b), the chemical shift of the nitronate carbon atom was δ 125.4 in $^2H_4$-methanol compared to δ 115.5 in $^2H_6$-DMSO - a difference of 9.9 p.p.m. (see Figures 11 and 12). In comparison, the solvent dependence of the chemical shift of this carbon in the parent nitro compound (2b) is considerably smaller (δ 90.1 in methanol, δ 89.1 in DMSO) (see Figures 13 and 14). Also in the nitronate anion (1) large long-range deshielding of carbon atoms by the nitronate group, analogous to those recorded in ^1H n.m.r. measurements, are not observed. Some small shift differences occur for C-2 and C-4 on anion formation, but these were not consistent with long-range deshielding and not all downfield (see Table 12). Assignment of these two carbon atoms was complicated by overlapping solvent signals when using methanol. In 2-bornylnitronate (28a), an example where ^1H n.m.r. measurements showed that the methyl substituent at C-1 lies in or near the plane of the nitronate group, there is no significant chemical shift difference for this methyl carbon (C-10) in the nitronate anion compared to the nitro compound (28) (see Figures 15 and 16). In $^2H_6$-DMSO, C-10 for (28) is at δ 13.8 and for (28a) at δ 13.5; in $^2H_4$-methanol the shieldings are δ 14.4 and δ 13.5 respectively. For both solvents C-10 moves slightly upfield on anion formation, confirming that the nitronate group does not appreciably affect the shielding of carbon atoms nearby in space. However, as observed for the anion (1), the chemical shift of the nitronate carbon atom in 2-bornylnitronate (28a) is particularly solvent dependent. In (28a) C-2 resonates at 13.4 p.p.m. to lower field in $^2H_4$-methanol than in $^2H_6$-DMSO (see Figures 17 and 18). Similar effects are observed in the ^{13}C spectra of other alkanenitronates; results are summarised in Table 13.

Table 13

Solvent Dependence of the Chemical Shift of the Nitronate Carbon Atom (0.5 M solution, 305° K)

Compound	δ(CD_3OD)	δ ($(CD_3)_2SO$)	δ CNO_2^- (CD_3OD) - δ CNO_2^- ($(CD_3)_2SO$)
(1) Sodium salt	125.4	115.5	9.9
Sodium 2-bornane nitronate	135.1	121.7	13.4
Lithium 1-propanenitronate	119.1	109.3	9.8
Lithium 2-propanenitronate	121.5	111.6	9.9
Sodium 2-propanenitronate	120.9	107.0	13.9

The complete assignments and shieldings are listed in Table 12.

It was found impossible to measure the chemical shift of the nitronate carbon in sodium-2-adamantyl nitronate due to low solubility in DMSO, and the apparently long relaxation time of this carbon atom compounded by the weak Nuclear Overhauser Effect produced by proton decoupling. Addition of a relaxing agent, $Cr(acac)_3$, did not facilitate observation of this signal. The low solubility of some sodium nitronate salts in DMSO prevented measurements in this solvent, although chemical shifts obtainable in methanol are recorded for completeness.

The Origin of ^{13}C Solvent Effects in Nitronate Anions and Comparison with Theories Proposed on the Basis of Electronic Spectral Evidence

From an investigation into the effect of solvents on anion structure, Kerber observed[138] that the ^1H n.m.r. and electronic spectra of salts of 1-nitroindene and 9-nitrofluorene showed significant changes between protic and aprotic solvents. It was concluded[138] that in protic solvents hydrogen bonding to the oxygen atoms of the nitronate tends to localise the negative charge on oxygen; analogous effects also being observed in phenoxide salts. This latter phenomenon had been recognised earlier by Brown.[139] In a study of the electronic spectra of substituted 1-phenyl-1-ethanenitronates, Shechter observed[140] similar effects. It was also claimed[140] that in the ultraviolet spectra of alkane nitronates, polar solvents of diminished hydrogen bonding ability caused absorption at longer wavelengths. For the examples quoted (sodium 2-propanenitronate and sodium ethanenitronate) the absorption maxima in methanol reported can be accurately reproduced. In this laboratory it has been impossible to reproduce the results given for alkane nitronates in acetonitrile or propylene carbonate, the only aprotic solvents examined, thus casting doubt on conclusions based on this data. The irreproducibility of these results arises because the nitronate salts quoted are too insoluble in acetonitrile or propylene carbonate for absorption measurements to be taken. Although Shechter's empirical reasoning is perfectly sound, the arguments are based on what appear to be fallacious results for alkane nitronates. Communication with Professor Shechter, in an attempt to resolve this discrepancy, has met with no response. Nonetheless, the theorised proposals are experimentally demonstrable, provided soluble nitronates are chosen. For example, the ultraviolet absorption maximum for the sodium nitronate (1) shows significant changes in the solvents methanol, acetonitrile and

propylene carbonate, absorption maxima being at 235 (ε 6,500), 248 (ε 4,900) and 259 (ε 4,400) nm respectively. The absorption difference to longer wavelengths represents a lessening of the $\pi - \pi^*$ transition energy and this is borne out by the decreased extinction coefficient as the absorption moves to longer wavelengths. Ultraviolet absorption data for ethanenitronate and 2-propanenitronate anions in methanol, DMSO and DMF have been reported,[141] and also demonstrate this marked solvent dependence. Some ultraviolet measurements for a number of aliphatic nitronates were made and are summarised in Table 14.

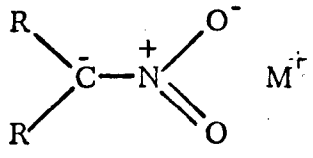
Table 14

Ultraviolet Absorption Maxima for Alkane Nitronates in Various Solvents

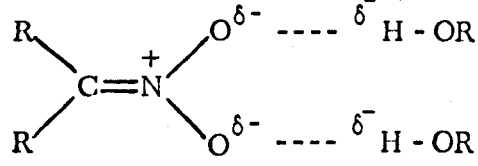
	MeOH	MeCN	Propylene Carbonate
Sodium ethanenitronate	232	Insoluble	Insoluble
Sodium 2-propanenitronate	228	Insoluble	Insoluble
Lithium 2-propanenitronate	227 (ε 17,700)	221 (ε 11,200)	-
Lithium 1-propanenitronate	235	Insoluble	-
Sodium cyclohexanenitronate	235	Insoluble	-
Lithium cyclohexanenitronate	237	Insoluble	-
Sodium 2-bornanenitronate	232	Insoluble	247
Sodium 2-adamantanenitronate	<220	Insoluble	-
Sodium salt (1)	235 (ε 6,500)	246 (ε 4,900)	259 (ε 4,400)

The limited solubility of the majority of the nitronates examined precludes the use of u.v. as a satisfactory technique for studying the structure of the nitronate group in various solvents. DMSO was unfortunately an unsuitable solvent due to its high ultraviolet cut-off at ca. 260 nm.

The ^{13}C n.m.r. measurements described above clearly uphold the suggestions[138] of Kerber, since the chemical shift differences observed for the nitronate carbon atom can be ascribed to the negative charge being localised more on carbon in DMSO, causing an increased shielding of this carbon atom. In methanol the negative charge is localised on oxygen, being stabilised by hydrogen bonding to the solvent. The changes in anion structure from dipolar aprotic to protic solvent is shown by structures (29) and (30).



(29)



(30)

^{17}O n.m.r. measurements[136] have also shown that in protic solvents such as methanol and water, the negative charge of aliphatic nitronate anions resides principally at the oxygen atoms. Anions derived from 2-nitropyrroles also demonstrate this behaviour.[142]

Factors Affecting Solvent-Nitronate Anion Interaction

The chemical shift of the nitronate carbon in methanol is not significantly changed by the concentration of base. For example, preparing anion (1) with one equivalent of base gave a value for $\underline{C}NO_2^-$ at δ 125.4; using two equivalents of base a corresponding value of δ 124.9 was obtained. The practical difficulties of varying the base concentration in DMSO by using sodium hydride precluded experiments to verify that the chemical shift of $\underline{C}NO_2^-$ was unaltered, but this would be a reasonable assumption.

Concentration effects

The effects of concentration on chemical shift were examined using lithium 2-propanenitronate. Although this nitronate salt is reasonably soluble in DMSO, it was not possible to use solutions of greater concentration than 1 M in this solvent. Significant chemical shift changes over the concentration range 0.25 - 1.0 M were not observed, as shown in Table 15.

Table 15

Effects of Nitronateanion Concentration on the ^{13}C Chemical Shifts of Lithium 2-Propanenitronate at 305° K

	δ CD_3OD		δ $(CD_3)_2SO$	
Concentration	$\underline{C}H_3$	$\underline{C}NO_2^-$	$\underline{C}H_3$	$\underline{C}NO_2^-$
0.25 M	19.0	121.5	18.2	111.6
0.5	19.0	121.5	18.2	111.7
1.0	19.0	121.4	18.3	111.9

For dilute solutions, the anion-solvent interaction would not be expected to vary

appreciably with anion concentration. The experimental results support this proposal.

Temperature effects

At a fixed concentration some interesting changes were recorded in the shielding of the nitronate carbon atom of lithium 2-propanenitronate on varying the temperature. These results are shown in Table 16.

Table 16

Effect of Temperature on ^{13}C Chemical Shifts in Lithium 2-Propanenitronate (0.5 M)

	δ (CD_3OD)			δ ($(CD_3)_2SO$)	
T^oK	$\underline{C}H_3$	$\underline{C}NO_2^-$	T^oK	$\underline{C}H_3$	$\underline{C}NO_2^-$
253	19.0	122.7	Solvent Crystallised $<291^oK$		
273	19.0	122.3	298	18.2	111.5
305	19.0	121.5	305	18.2	111.7
323	19.0	121.2	323	18.1	112.0

Although the chemical shift of the nitronate carbon shows only small changes at differing temperatures, the results exhibit definite trends. In methanol, a lowering of the temperature causes a downfield shift on $\underline{C}NO_2^-$ of about 0.55 p.p.m./25^o and in DMSO similar temperature changes cause an upfield shift of about 0.5 p.p.m./25^o. By contrast, the chemical shift of the methyl carbon is unaffected in either solvent. Again, these effects can be explained in terms of the hydrogen bonding ability of the solvent. Cooling methanolic solutions causes an increase in anion-solvent interaction by slowing down the rate of exchange of solvent molecules, whereby the negative charge is even more stabilised on the oxygen atoms of the nitronate. In DMSO, cooling causes an increase in the solvation of the cation, causing an even greater proportion of the negative charge to reside on the nitronate anion, resulting in greater shielding of the nitronate carbon atom.

Another phenomenon observed to be both solvent and temperature dependent, was the relaxation time T_1. It is known that T_1 is normally dependent on temperature, being shortened at lower temperatures. The $\underline{C}NO_2^-$ signal in most of the nitronates is of low intensity due to both weak NOE and a long relaxation time T_1. In lithium 2-propanenitronate at ambient temperatures, the $\underline{C}NO_2^-$ signal was not observable when using a long pulse width (20 μS) (25 μS ≈ 90^o flip angle). A short pulse width of

7 - 10 μS was optimum. On cooling a methanolic solution of lithium 2-propanenitronate, for a given number of accumulations, the $\underline{C}NO_2^-$ signal noticeably increased. This indicates a shortening of T_1 caused by increased relaxation processes via the solvent (no absolute T_1 measurements were made). In DMSO, where less anion-solvent interaction is suggested, little change in T_1 occurred with changing temperature. An extensive study of temperature effects in other nitronate anions was not carried out, but the observations discussed above are expected to be general.

Effect of the Counter-ion

On the basis of infrared spectral evidence, it has been concluded[140] that lithium alkanenitronate salts are more covalent than the corresponding sodium and potassium salts, since shifts to longer wavelengths were observed as the charge density on the cation decreased.

From a limited amount of data, ^{13}C n.m.r. evidence offers support for this theory. Comparing the ^{13}C chemical shifts for sodium 2-propanenitronate and lithium 2-propanenitronate shows little difference. (Lithium salt : δ $\underline{C}H_3$ 19.0; $\underline{C}NO_2^-$ 121.5; sodium salt : δ $\underline{C}H_3$ 19.0; $\underline{C}NO_2^-$ 121.0). In methanol the anion is probably so heavily solvated that the cation has little bonding influence and therefore a similar effect on carbon atoms in the anion. In DMSO however, one would expect the differences to be greater. This is the case, and in DMSO $\underline{C}NO_2^-$ appears at δ 111.6 for lithium 2-propanenitronate and for sodium 2-propanenitronate at δ 107.0. This may be rationalised as follows. Because of tighter coordination of the nitronate oxygen atoms with a cation of relatively high charge density, the lithium nitronate salts are more covalent, resulting in a diminished charge density on the anion. This causes less shielding of the nitronate carbon than is the case for the sodium nitronate salt, which has greater charge separation, being more ionic. The resultant increased electron density of the anion causes greater shielding of the nitronate carbon atom, which is consequently observed at a higher chemical shift than for the lithium nitronate.

Alkyl Substituents on the Nitronate Carbon Atom

It is appropriate that some comment be made on the effect of alkyl groups attached to the nitronate carbon atom, compared with hydrogen substituents. Results are, at present, limited due to the very low solubility of the available primary sodium alkanenitronates in DMSO. Also in these salts, the hydrogen atom attached to the

nitronate carbon quickly exchanges with deuterium in $^{2}H_{4}$-methanol (confirmed by ^{1}H n.m.r.) and it was impossible to observe the ^{13}C signal for the nitronate carbon due to the diminished NOE and the signal broadening caused by C - D coupling. The use of CH_3OH containing a small proportion of $^{2}H_{4}$-methanol resolved this problem. (The deuterated solvent was required for the deuterium lock signal).

Nevertheless these results enable some comment to be made on the hypothesis[140] concerning the ground state structure of nitronate anions based on measurements of ionisation constants in polar protic solvents such as water or methanol. The ^{13}C n.m.r. observations are also consistent with data presented for the levelling influence of DMSO on the acidity of mononitroalkanes.[141]

In polar protic solvents the ionisation constants of nitroalkanes increase in the following order : $(CH_3)_2CHNO_2 > CH_3CH_2NO_2 > CH_3NO_2$ (the pKa's are in the reverse order), whereas in DMSO the pKa's are similar, although the nitro compounds display a much weaker acidity in this solvent. It was suggested[140] that in protic solvents 2-propanenitronate anion is relatively highly hydrogen bonded on oxygen, causing less carbanionic character at the nitronate carbon. Ethanenitronate, being less highly hydrogen bonded, displays relatively more carbanionic character at C-1. A comparison of the ^{13}C shieldings for sodium ethanenitronate and sodium 2-propanenitronate in methanol verify this argument. The nitronate carbon in ethanenitronate is significantly more shielded (δ 112.3) than for this carbon in 2-propanenitronate (δ 121.0), although the additional methyl group in the latter accounts for some of the difference. Methanenitronate anion is relatively poorly hydrogen bonded and due to less extensive solvation, should exhibit more carbanion character. This is reflected in a shielding of δ 109.8 for the nitronate carbon atom.

The low solubility of primary alkane nitronates in DMSO prevented their examination.

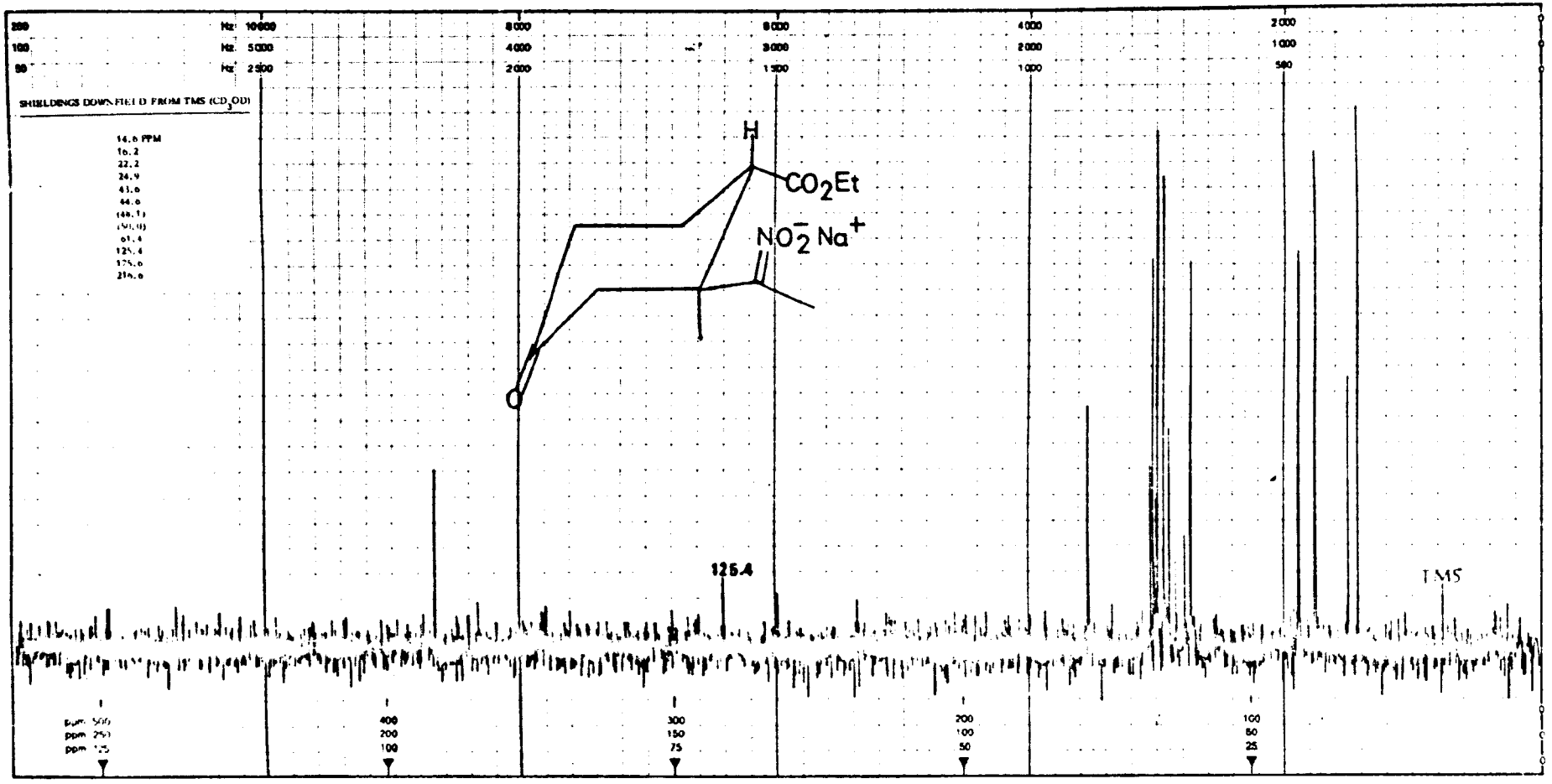


Figure 11 22.63 MHz(CD3OD) ^{13}C nmr spectrum of 4-Ethoxycarbonyl-3-methyl-3-(sodium 2'-ethylnitronate) cyclohexanone

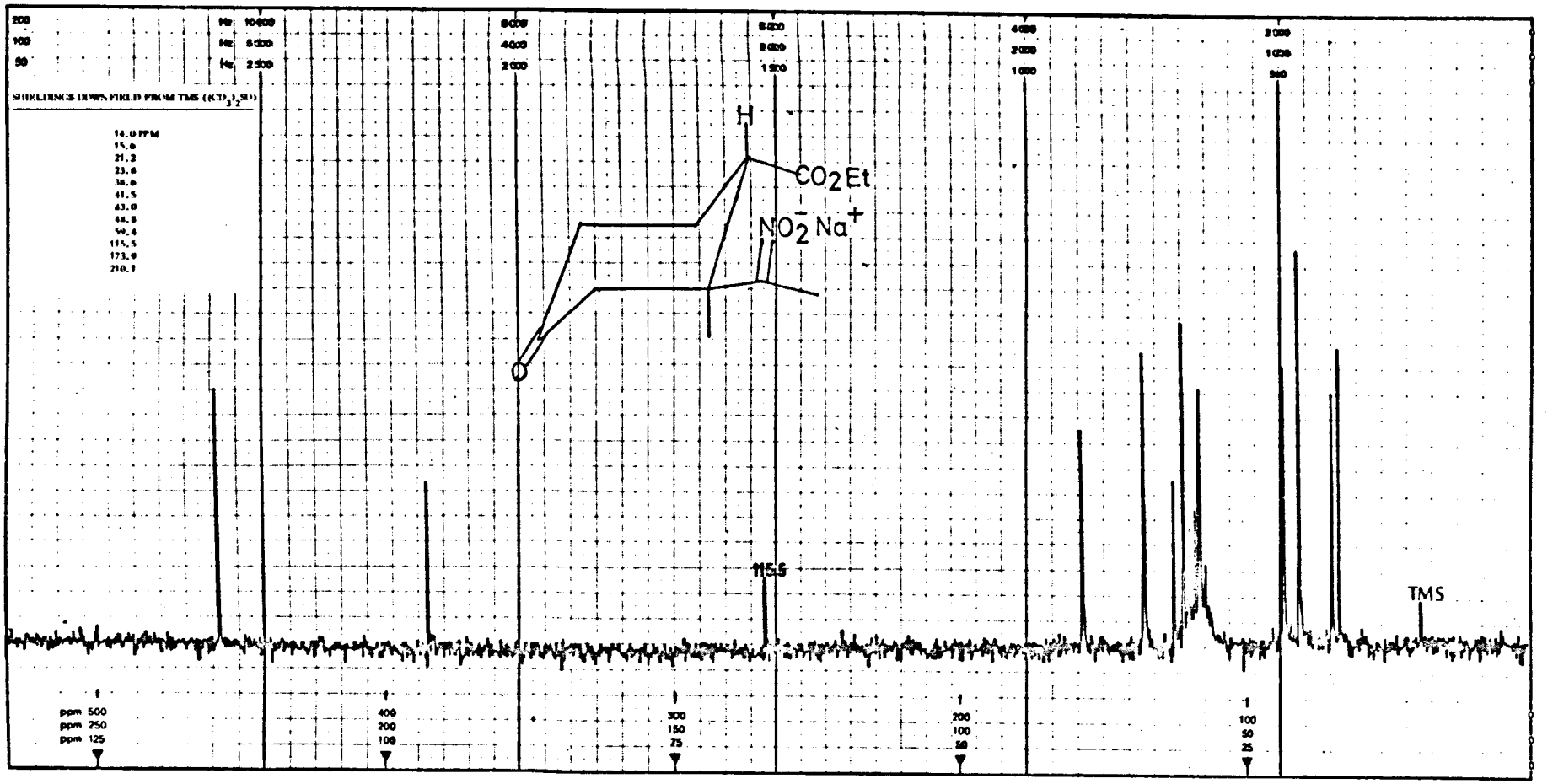


Figure 12 22.63 MHz((CD3)2SO) ^{13}C nmr spectrum of 4-Ethoxycarbonyl-3-methyl-3-(sodium 2'-ethylnitronate) cyclohexanone

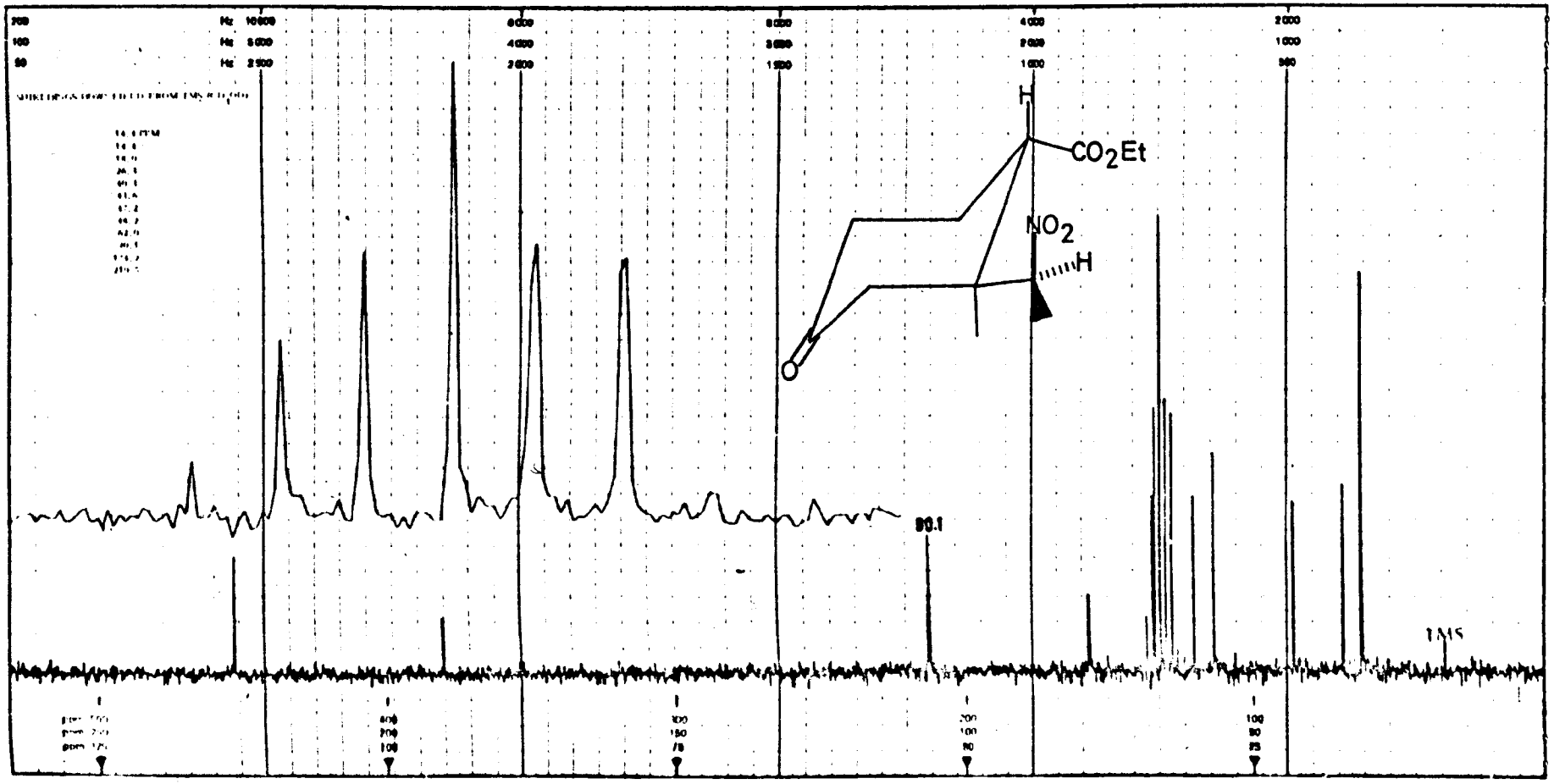


Figure 13 22.63 MHz(CD3OD) ^{13}C nmr spectrum of 4-Ethoxycarbonyl-3-methyl-3-(2'-nitroethyl) cyclohexanone

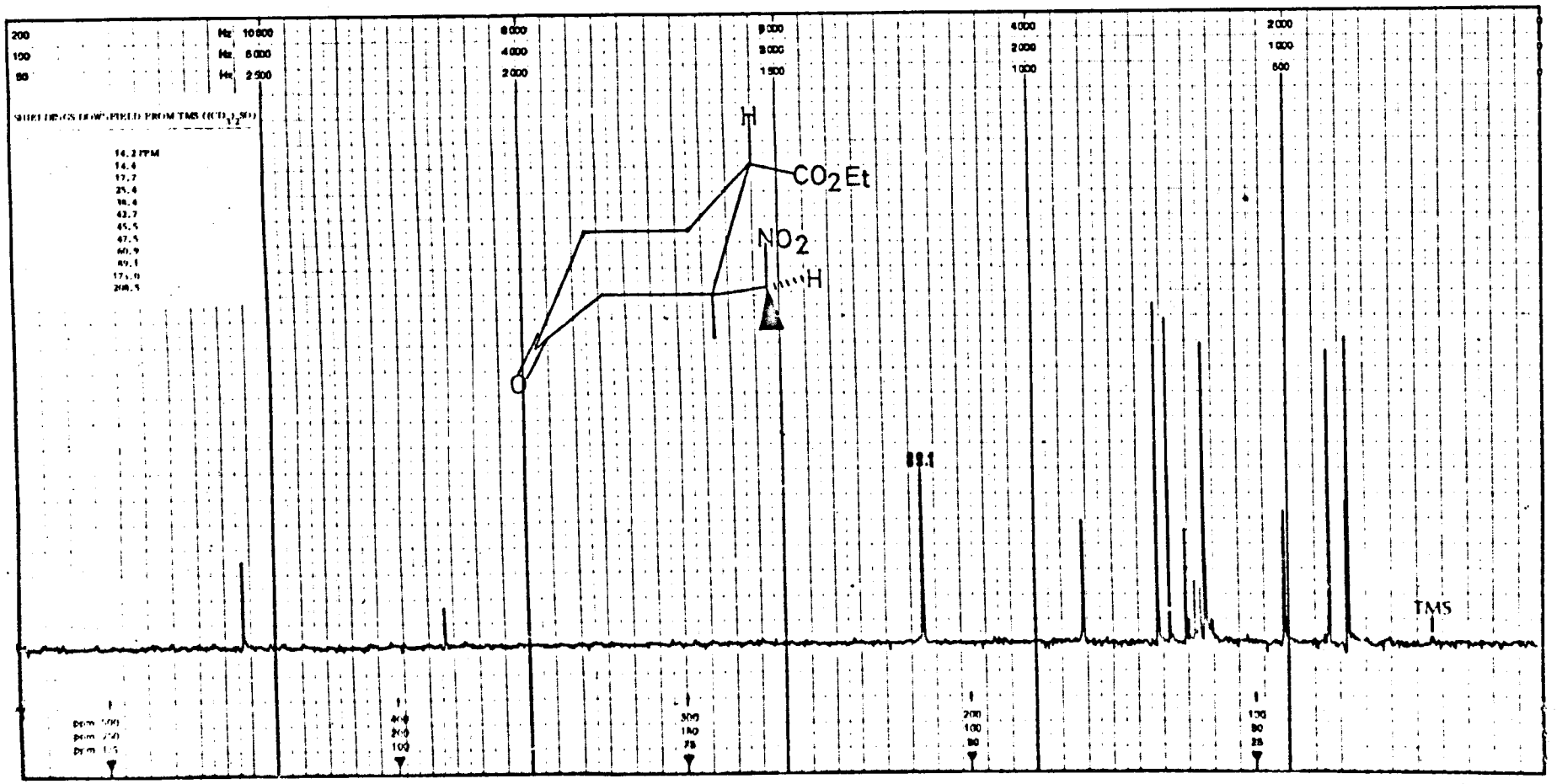


Figure 14 22.63 MHz((CD3)2SO) ^{13}C nmr spectrum of 4-Ethoxycarbonyl-3-methyl-(2'-nitroethyl) cyclohexanone

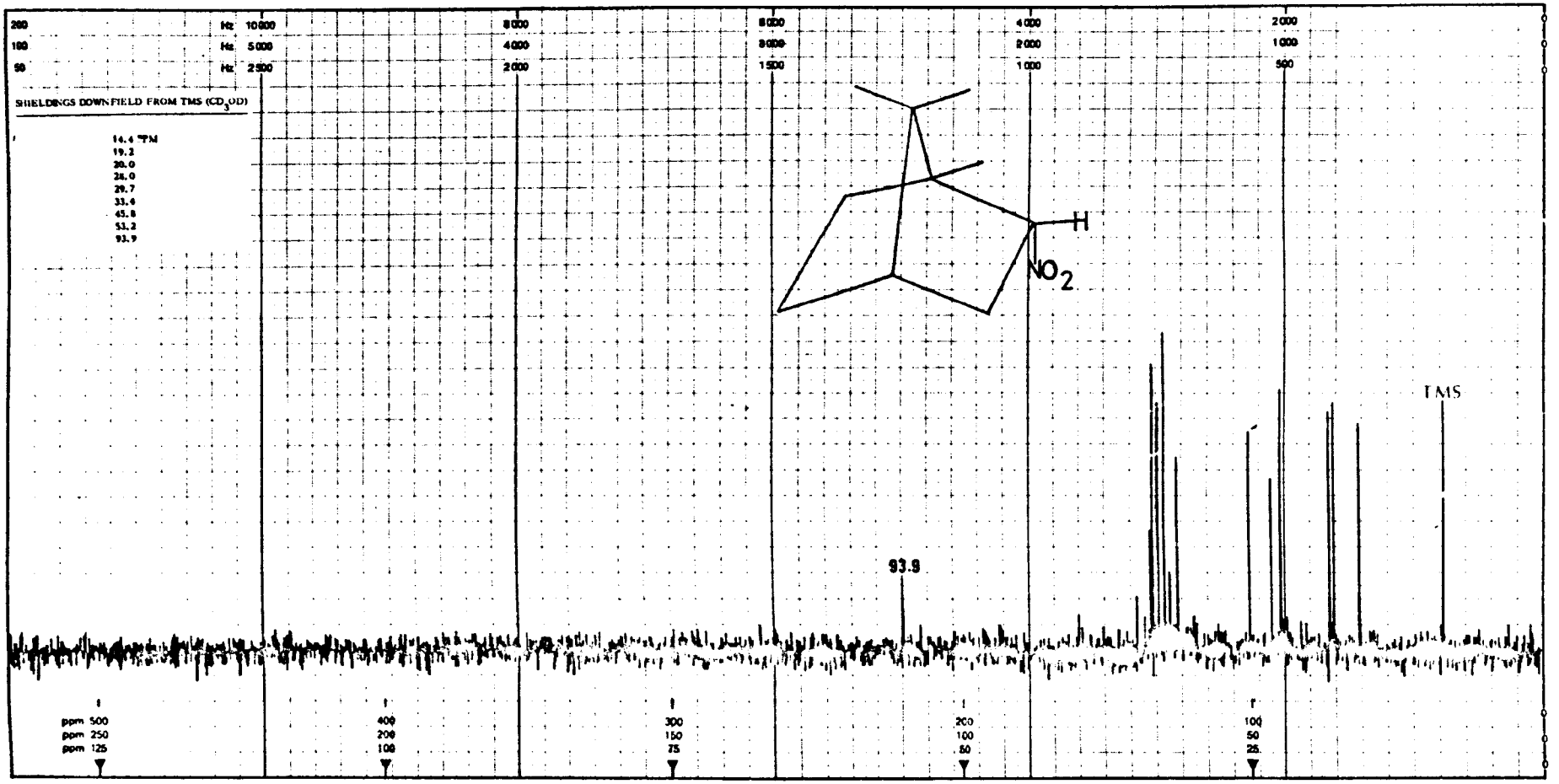


Figure 15 22.63 MHz(CD_3OD) ^{13}C nmr spectrum of 2-Nitrobornane

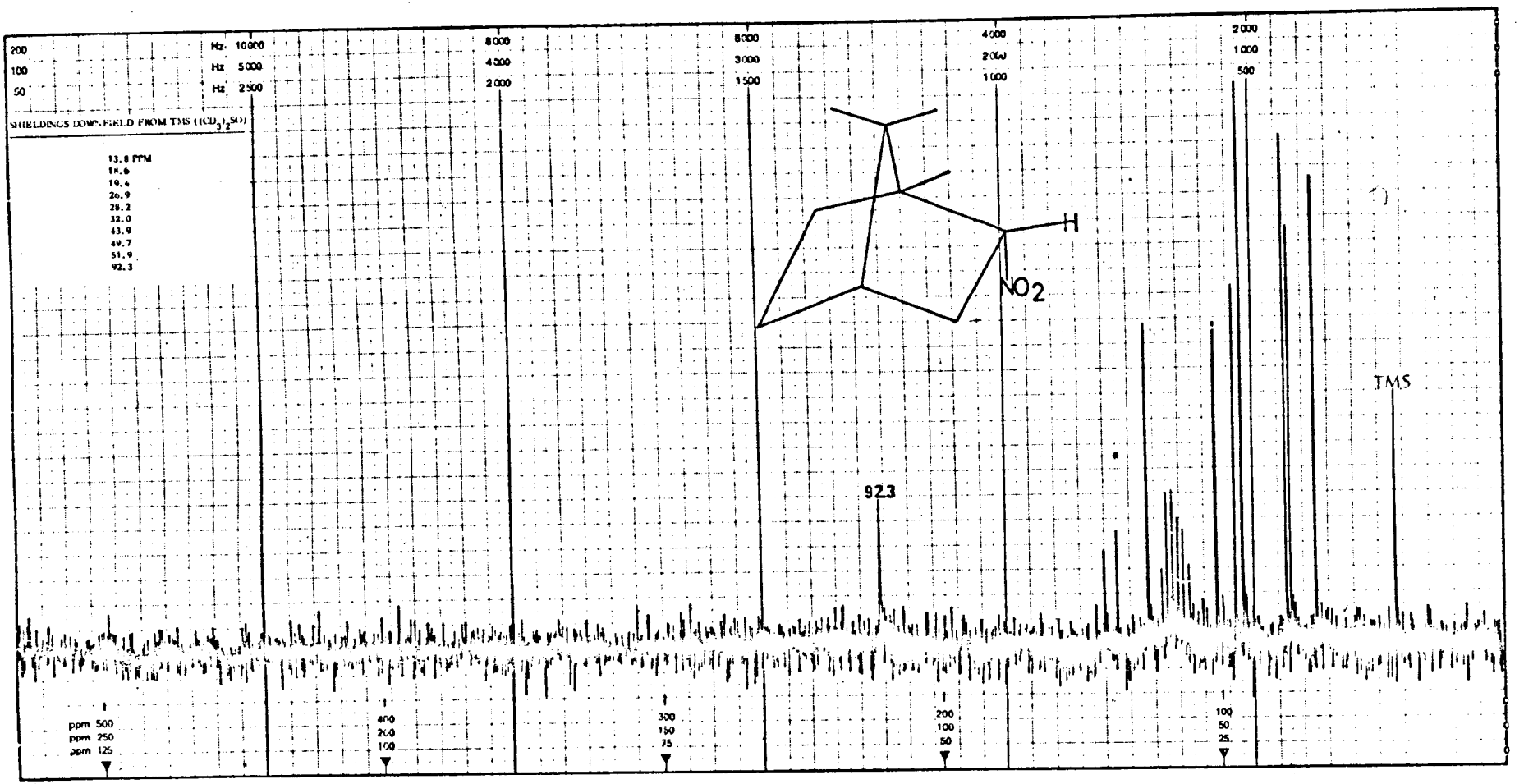


Figure 16 22.63 MHz((CD3)2SO) ^{13}C nmr spectrum of 2-Nitrobornane

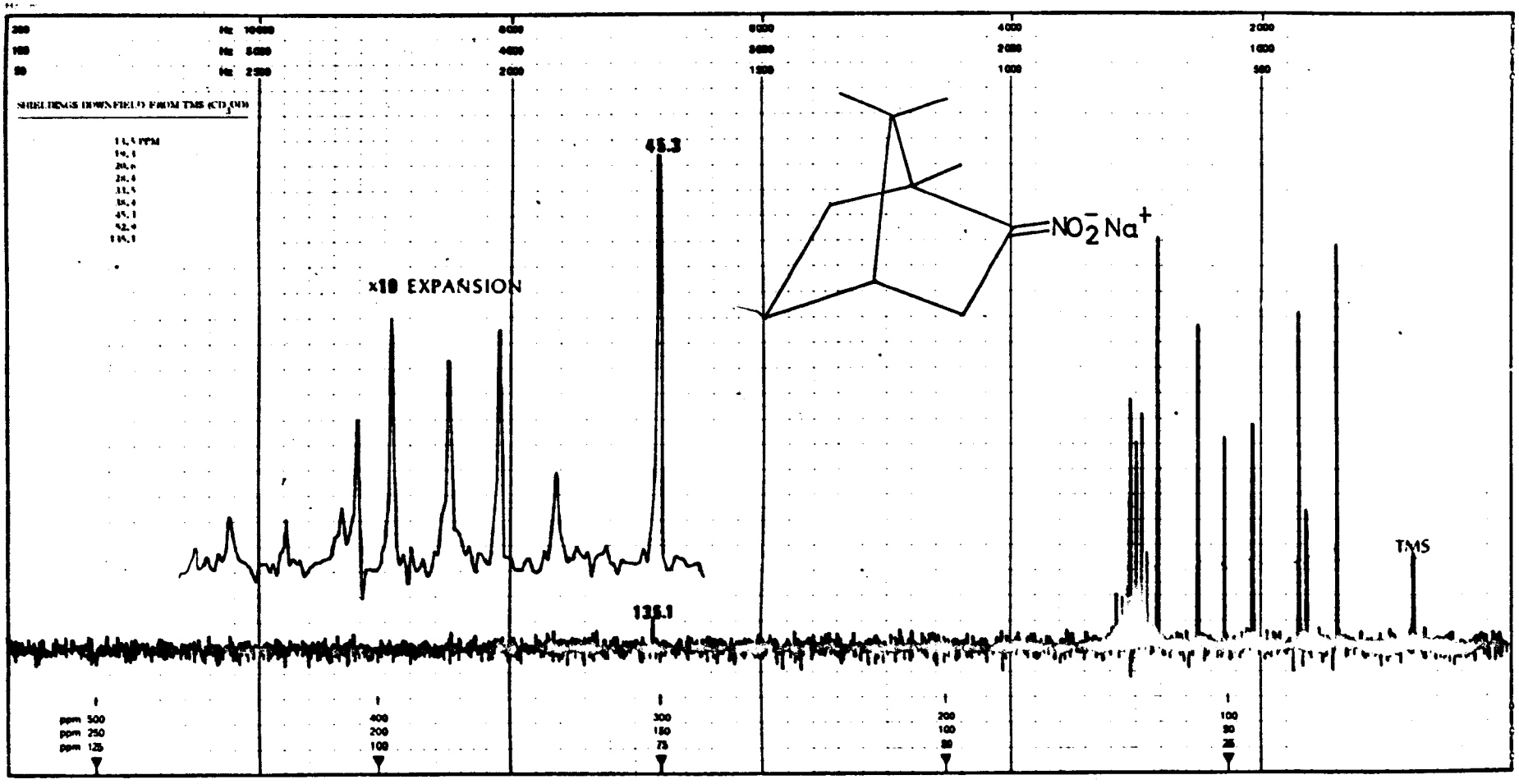


Figure 17 22.63 MHz(CD3OD) ^{13}C nmr spectrum of Sodium 2-bornyl nitronate

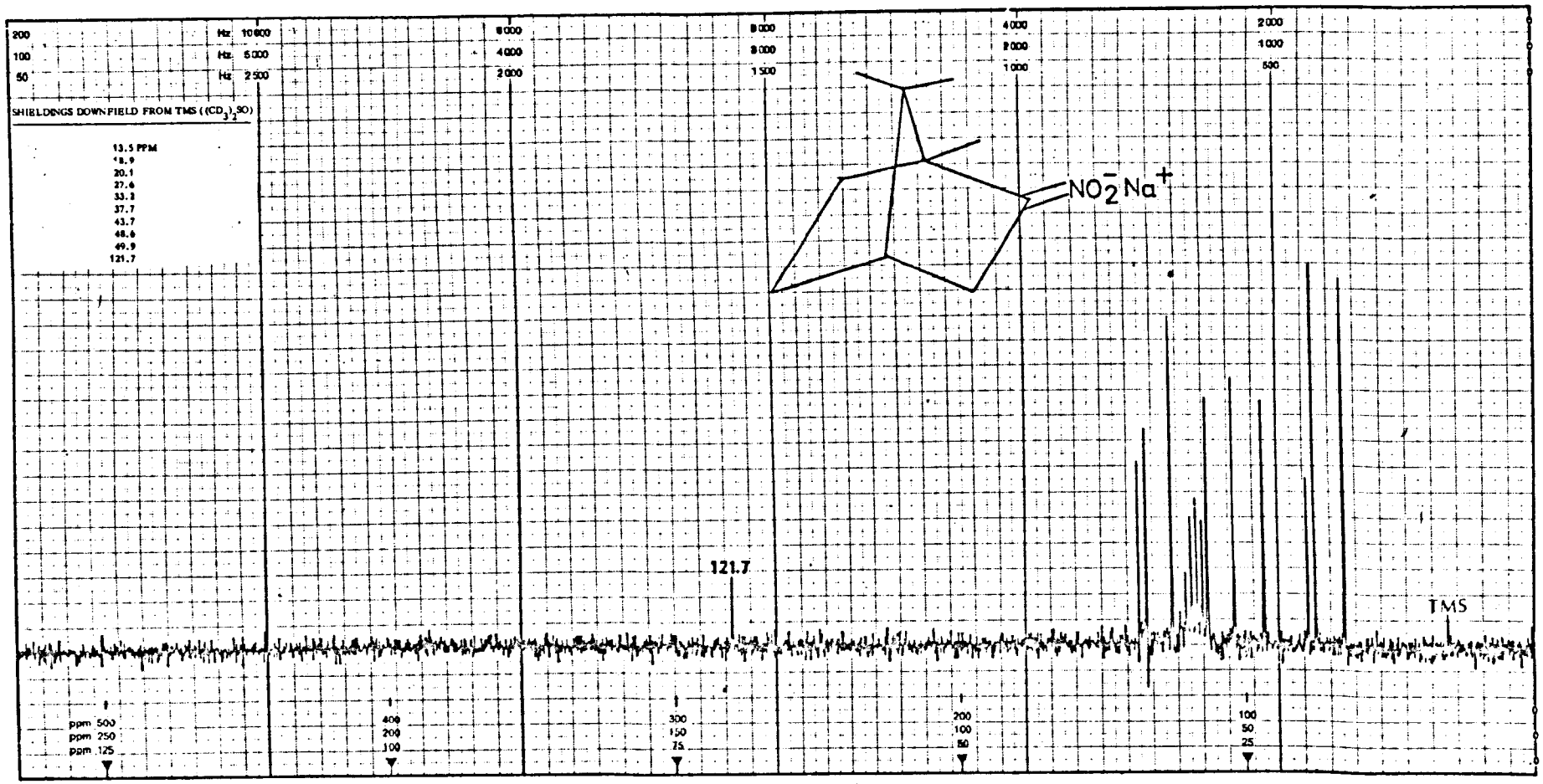


Figure 18 22.63 MHz((CD3)2SO) ^{13}C nmr spectrum of Sodium 2-bornyl nitronate

EXPERIMENTAL IV

Materials

Ring A Precursors

The keto nitroethyl ester (2) was prepared by Michael addition[17] of nitroethane to ethyl Hagemann's ester catalysed by benzyltrimethylammoniumethoxide as described in Chapter 1 (page 36). The t-butyl ester (7) was similarly prepared[17] from t-butyl Hagemann's ester[16] using benzyltrimethylammonium t-butoxide as catalyst. The ethylene ketal nitroethyl ester (9) was prepared as described in Chapter 1 (page 37).

Nitro Compounds

2-Nitroadamantane, nitrocyclohexane, 2-phenylnitrocyclohexane, 1-nitro-trans-decalin, 2-nitronorbornane and 2-nitrobornane were prepared from the corresponding oximes using the Iffland procedure.[56] They were purified by either recrystallising, distilling or subliming in vacuo where appropriate.

2-Nitroadamantane is previously unreported and was best purified by chromatography on silica gel, eluting with 9 : 1 CH_2Cl_2/petrol (40 - 60°), followed by sublimation in vacuo (70°C/15 mm) to give analytically pure material.

m.p. : 179 - 180°C

n.m.r. : Table 5

i.r. (CH_2Cl_2) : νcm^{-1} 2922 s, 2860 s, 1550 - 1535 vs, 1454 s

		%C	H	N
$C_{10}H_{15}NO_2$	requires:	66.27	8.34	7.73
	found:	66.18	8.00	7.77

2-Phenyl-1-nitrocyclohexane was pure cis-isomer m.p. 58° (lit.[123] 58 - 58.5°).

The 1-nitro-trans-decalin isolated was a mixture of trans-trans and trans-cis 1-nitrodecalins which was distilled in vacuo (b.p. 82°/0.75 mm).

i.r. (liq.film) : νcm^{-1} 2920 s, 2850 s, 1550 s, 1450 s

		%C	H	N
$C_{10}H_{17}NO_2$	requires:	65.54	9.35	7.64
	found:	65.35	9.07	7.61

Pure trans-trans-1-nitrodecalin was obtained by stirring the mixture of trans-cis isomers in EtOH with a catalytic quantity (5 mole %) of NaOEt for 20 hours. EtOH was removed in vacuo and CH_2Cl_2 added to the residue; filtration and evaporation gave a single epimer.

The Emmons oxidation procedure[143] was used for preparing 2-methylnitro-

cyclohexane (and 2-nitroadamantane). 2-Methylnitrocyclohexane as prepared was 80% cis-isomer.[144]

Nitromethane, nitroethane, 1-nitropropane and 2-nitropropane were commercially available and were distilled before use. Pure solvent-free nitronate salts were prepared using the general method described by Kornblum.[57] Solvents were purified and dried according to literature methods described by Perrin.[145]

N.M.R. Measurements

Deuterated solvents were dried with freshly activated molecular sieves (3 Å), deoxygenated with, and stored under argon in bottles fitted with a rubber septum. ^{1}H n.m.r. spectra for 2-nitroadamantane, 2-phenylnitrocyclohexane, 2-nitronorbornane and their corresponding nitronate salts were recorded at 100 MHz using a Varian HA-100 spectrometer. For nitrocyclohexane, 2-methylcyclohexane, 2-nitrobornane, the keto nitroethyl ester (2), the ethylene ketal nitroethyl ester (9) and their nitronate anions, measurements were made at 100 MHz using a JEOL MH-100 spectrometer. The spectra for (2) and the t-butyl analogue (7) and their nitronates in $(CD_3)_2SO$ were recorded at 60 MHz using a Perkin Elmer R12. A Bruker WH 90 spectrometer was used for recording the spectra of trans-trans-1-nitrodecalin and its nitronate anion at 90 MHz using the pulse Fourier Transform method. Solutions were 0.2 - 0.4 M in concentration and TMS (δ 0.00) was used as internal reference. Deprotonations of the nitro compounds in CD_3OD were performed by addition of 1.1 equivalents of $NaOCD_3$ in CD_3OD (prepared by dissolving clean sodium in CD_3OD and standardising with dilute HCl) under argon. Spectra were recorded, followed by addition of a second equivalent of base to ensure complete deprotonation and to observe whether chemical shifts were dependent on base concentration. Trans-1-decalylnitronate was examined by dissolving a sample of the pre-prepared nitronate salt. Deprotonations in $(CD_3)_2SO$ were carried out by addition of 1.1 equivalents of NaH to solutions of the nitro compounds under an inert atmosphere. (NaH was prepared by washing a 50% dispersion in oil with dry pentane in a dry N_2 glove box). When effervescence ceased, the spectra were recorded.

^{13}C n.m.r. spectra were recorded at 22.63 MHz for approximately 0.5 M solutions (except where noted otherwise for experiments examining concentration dependence) in CD_3OD or $(CD_3)_2SO$ in 10 mm diameter sample tubes by the pulse Fourier Transform technique. A Bruker WH 90 spectrometer was used with 4K or 8K of data storage for accumulation of the free induction decays. The noise

modulated proton decoupling was sufficient to decouple the complete range of proton-carbon coupling, and pulses of approximately 25 - 30° (7 - 10 μS) were used. To confirm certain assignments, where possible, the single frequency off resonance decoupling technique was used.

The spectra for nitronate anions were recorded by either addition of 1.1 equivalents of base ($NaOCD_3$ or NaH) to the nitro compound in the appropriate solvent, or by dissolving pure pre-prepared nitronate salts, all manipulations being carried out under an inert atmosphere. For primary alkanenitronates, a 4:1 mixture of CH_3OH / CD_3OD was used, the deuterated solvent being for the deuterium locking signal. Chemical shifts were measured downfield from TMS (δ 0.00).

Ultraviolet Spectra

The wavelengths of maximum absorption in the ultraviolet spectra of nitronate anions were determined by dissolving pure nitronate salts in the appropriate solvent. Measurements were made using a Pye Unicam SP 800 spectrophotometer, calibrated with a Holmium filter. Where extinction coefficients varied, the highest value obtained was recorded.

Solvents were purified before use. Methanol was spectroscopic grade and was dried by refluxing with sodium, then distilled under inert atmosphere. (N.B. commercial spectroscopic grade methanol contained impurities which reacted with the nitronates; the above treatment was a satisfactory method for their removal). Acetonitrile was spectroscopic grade and was stirred with CaH_2 for 24 hours, decanted, then distilled under an inert atmosphere. Propylene carbonate was stirred with molecular sieves (3 Å) for 24 hours, then fractionally distilled in vacuo. All solvents were stored under argon in bottles fitted with a rubber septum. Prior to use, solvents were deoxygenated with argon. Ultraviolet spectral measurements were made immediately after making up the nitronate solutions.

CHAPTER V

Synthesis and Preliminary Study of New Substrates for Glyceroldehydrase

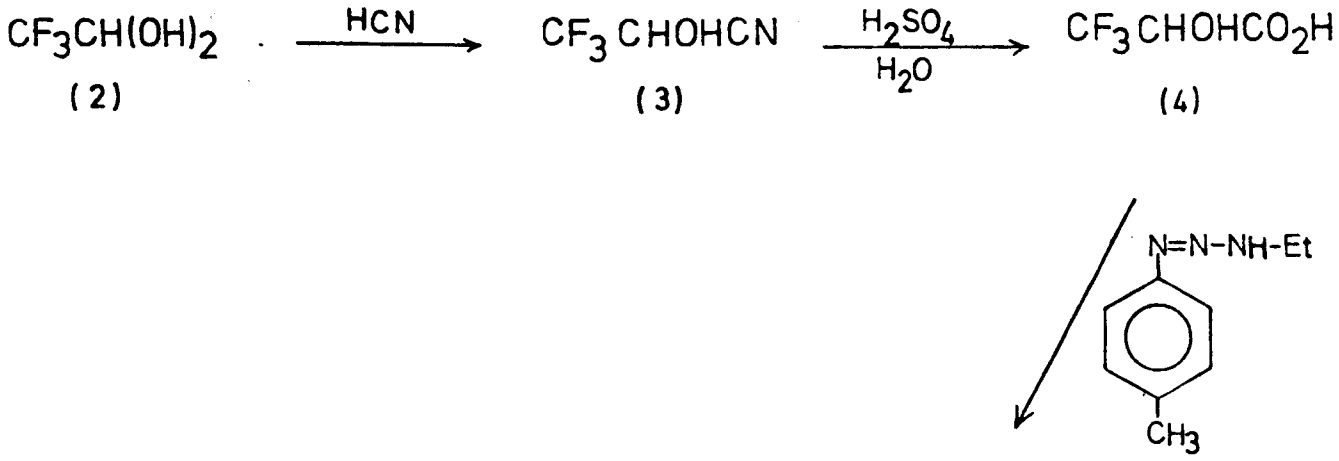
Structurally, 5'-deoxyadenosyl cobalamin is probably the most complex coenzyme in nature, and as discussed in the introductory chapter, enzymes requiring this cofactor have stimulated considerable interest. The following describes the author's contribution, in collaboration with members of the Oxford Enzyme Group, investigating the mechanism of glyceroldehydrase, an enzyme extracted from A. Aerogenes PZH 572(Warsaw). The synthetic studies concerning potential substrates, and products derived from enzymatic reactions have been carried out at Warwick. The enzymology and nuclear magnetic resonance studies were undertaken by Dr M.A. Foster, Dr O.D. Hensens, Dr H.A.O. Hill and Professor R.J.P. Williams. The results of their work are discussed to add coherence to the text.

Although intensive research efforts have elucidated the fate of the hydrogen atoms during the enzymatic dehydration of ethanediol and 1,2-propanediol, the complete mechanism of the reaction is still subject to considerable speculation (See Introduction). The discovery of new substrates for a particular enzyme is always of great assistance in adding to the overall knowledge as to how the substrate interacts with the enzyme (or coenzyme). In this study, the initial objective was to synthesise 3,3,3-trifluoro-1,2-propanediol (1) and to examine its reactions with glyceroldehydrase. The fluorine atoms in this compound offer several possibilities for gaining information about intermediates in the enzymatic reaction. ^{19}F n.m.r. studies might be helpful, and since radical intermediates had been observed during the enzymatic dehydration of 1,2-propanediol, it was anticipated that fluorinated radicals might allow identification of intermediates from their e.s.r. signals. Fortunately, R,S-3,3,3-trifluoro-1,2-propanediol was a substrate for the enzyme, being converted to 3,3,3-trifluoropropionaldehyde which was identified as its 2,4-dinitrophenylhydrazine derivative. Interestingly, the reaction was not completely straightforward, and some unusual observations are discussed (see below).

Synthetic Studies

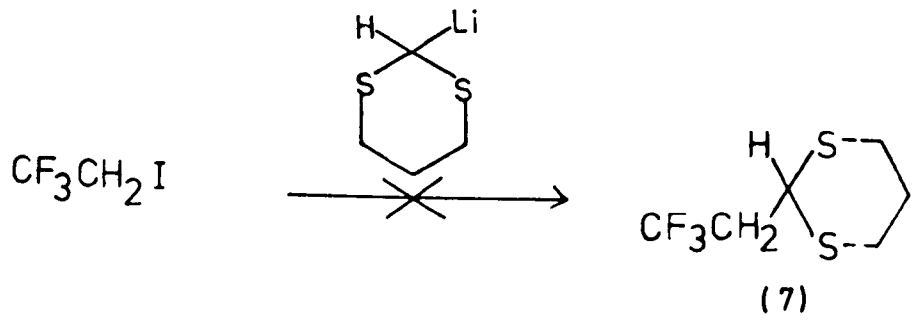
R,S-3,3,3-Trifluoro-1,2-propanediol

3,3,3-Trifluoro-1,2-propanediol had been prepared by McBee[146], starting

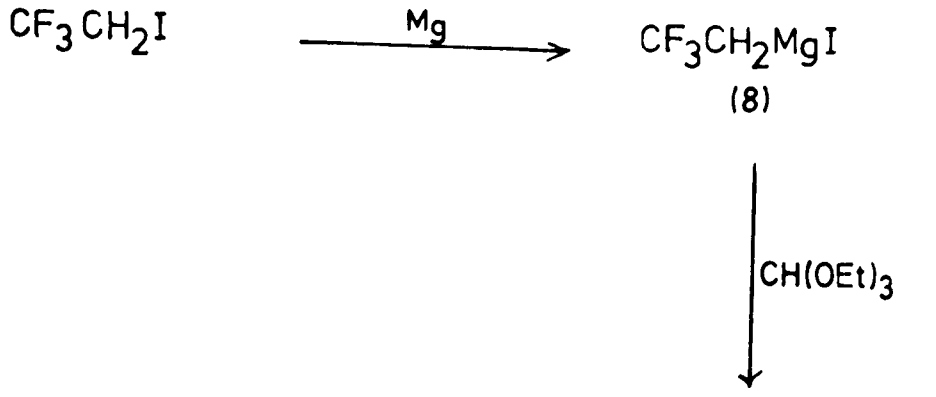

CF3CH(OH)2
(2)
HCN
CF3CHOHCN
(3)
H2SO4
H2O
CF3CHOHCO2H
(4)
N=N-NH-Et
CH3
CF3CHOHCH2OH
(1)
LiAlH4
CF3CHOHCO2Et
(5)

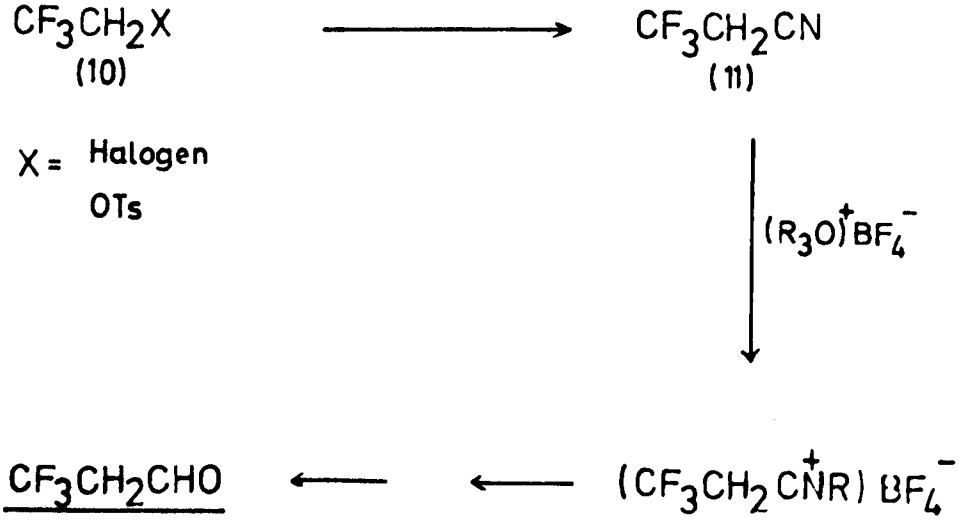
from 3-bromo-1, 1, 1-trifluoro-2-propanone. His method involved reduction of this ketone to the corresponding bromo alcohol which was converted to 3, 3, 3-trifluoro-1, 2-epoxypropane. Some 3, 3, 3-trifluoropropane-1, 2-diol was also produced during the dehydrohalogenation of the bromohydrin. Aqueous acid slowly hydrolysed the trifluoroepoxypropane to the trifluorodiol in low yield. Although this method was satisfactory, procedures starting from other readily available materials were examined. 3, 3, 3-trifluorolactic acid (4) had been synthesised[147] for evaluation in a search for inhibitors of lactic acid dehydrogenase. It was prepared by the reaction of sodium cyanide-sulphuric acid with 2, 2, 2-trifluoro-acetaldehyde hydrate (2), and the intermediate fluoral cyanohydrin (3) was then hydrolysed to the racemic acid. Unaccountably, when we carried out the reaction between trifluoroacetaldehyde and hydrogen cyanide on a ca. 0.2 M scale, several experiments resulted[148] in severe polymerisation, although it is known[147] that fluoral cyanohydrin polymerises at room temperature within a week. Small scale experiments reproduceably gave good yields of the cyanohydrin; McBee had reported[149] a reaction on a 0.5 M scale but the yield was low. The fluoral cyanohydrin was readily hydrolysed following the published procedure[147] to 3, 3, 3-trifluorolactic acid in high yield.

Esterification of the trifluorolactic acid was easily accomplished. Refluxing the acid with ethanol and a catalytic quantity of sulphuric acid and removal of water by azeotropic distillation successfully gave[148] 3, 3, 3-trifluoroethyllactate (5). A convenient alternative was to react the trifluorolactic acid with 1-ethyl-3-p-toluene-triazene in ether. The reaction was extremely rapid and was complete within 30 minutes, giving a good yield of the ester which was crystalline, but extremely volatile. Reduction of the ester using lithium aluminium hydride was effective for preparing the 3, 3, 3-trifluoro-1, 2-propanediol. An identical product resulted from the reduction of the ethyl trifluorolactate produced by esterification using ethanol. Some difficulties were encountered with products derived from procedures involving 1-ethyl-3-p-toluenetriazene. Although the ethyl trifluorolactate prepared using this reagent was purified by treatment with activated charcoal and was then pure by n.m.r., the colourless crystals yellowed on storage. Because of the volatility of this compound, reduction was carried out directly. Distillation of the resulting trifluoro-propanediol caused some polymerisation which may have been catalysed by trace impurities. Trifluoropropanediol arising from ethyl trifluorolactate prepared by the alternative procedure, distilled satisfactorily. Conditions for maximum yield


H
Li
S
S
CF_3CH_2I
H
S
CF_3CH_2
S

(7)


CF_3CH_2I
Mg
CF_3CH_2MgI
(8)
$CH(OEt)_3$
CF_3CH_2CHO
(6)
$CF_3CH_2CH(OEt)_2$
(9)


CF_3CH_2X
(10)
X = Halogen
OTs
CF_3CH_2CN
(11)
$(R_3O)^+BF_4^-$
CF_3CH_2CHO
$(CF_3CH_2\overset{+}{C}NR)\,BF_4^-$

of trifluoropropanediol have yet to be optimised, although the handling problems of ethyl-3, 3, 3-trifluorolactate could no doubt be overcome be preparing an ester of higher molecular weight.

3, 3, 3-Trifluoropropionaldehyde (6)

On finding that 3, 3, 3-trifluoro-1, 2-propanediol is a substrate for glycerol-dehydrase, being converted to 3, 3, 3-trifluoropropionaldehyde, it was necessary to determine the effects of the aldehyde on the enzyme. For this purpose we had to synthesise a pure authentic sample of trifluoropropionaldehyde from readily available materials. 3, 3, 3-trifluoropropionaldehyde has been prepared[150] by dichromate oxidation of the corresponding alcohol. The effects of the trifluoro-methyl group on other methods of aldehyde synthesis have not been previously examined. The following experiments illustrate the unusual reactivity of trifluoro-methyl compounds.

2-Lithium-1, 3-dithianes

The dithiane method is a convenient route for the preparation of aldehydes[87a, 151]. Lithium-1, 3-dithiane is reacted with, for example, an alkyl halide and the resulting substituted dithiane is hydrolysed in the presence of mercuric ions to the aldehyde. The trifluoromethyl groups of 2, 2, 2-trifluoroethyliodide deactivated this compound so that it did not undergo normal S_N2 displacement of the iodine by the nucleophilic dithiane anion. Rather, it appeared that the trifluoroethyl iodide reprotonated the lithium dithiane since very little acid was required for neutralisation at the end of the reaction and 1, 3-dithiane could be recovered quantitively. Several other (minor) products were observed on t.l.c., but these were not identified. A possible complexity was dehydrofluorination of products, leading to vinylogous difluoro compounds.

Grignard Reagents

Grignard reagents react with triethyl orthoformate and the resulting diethyl acetals can be hydrolysed to aldehydes[152]. Using this method, McBee prepared[153] 4, 4, 4-trifluorobutyraldehyde from the magnesium Grignard of 3-chloro-1, 1, 1-trifluoropropane. 2, 2, 2-Trifluoroethyliodide reacted only very slowly with magnesium. The reaction did not appear to be satisfactory, as the Grignard reagent formed evolved a gas. The crude Grignard reagent was reacted with triethyl orthoformate

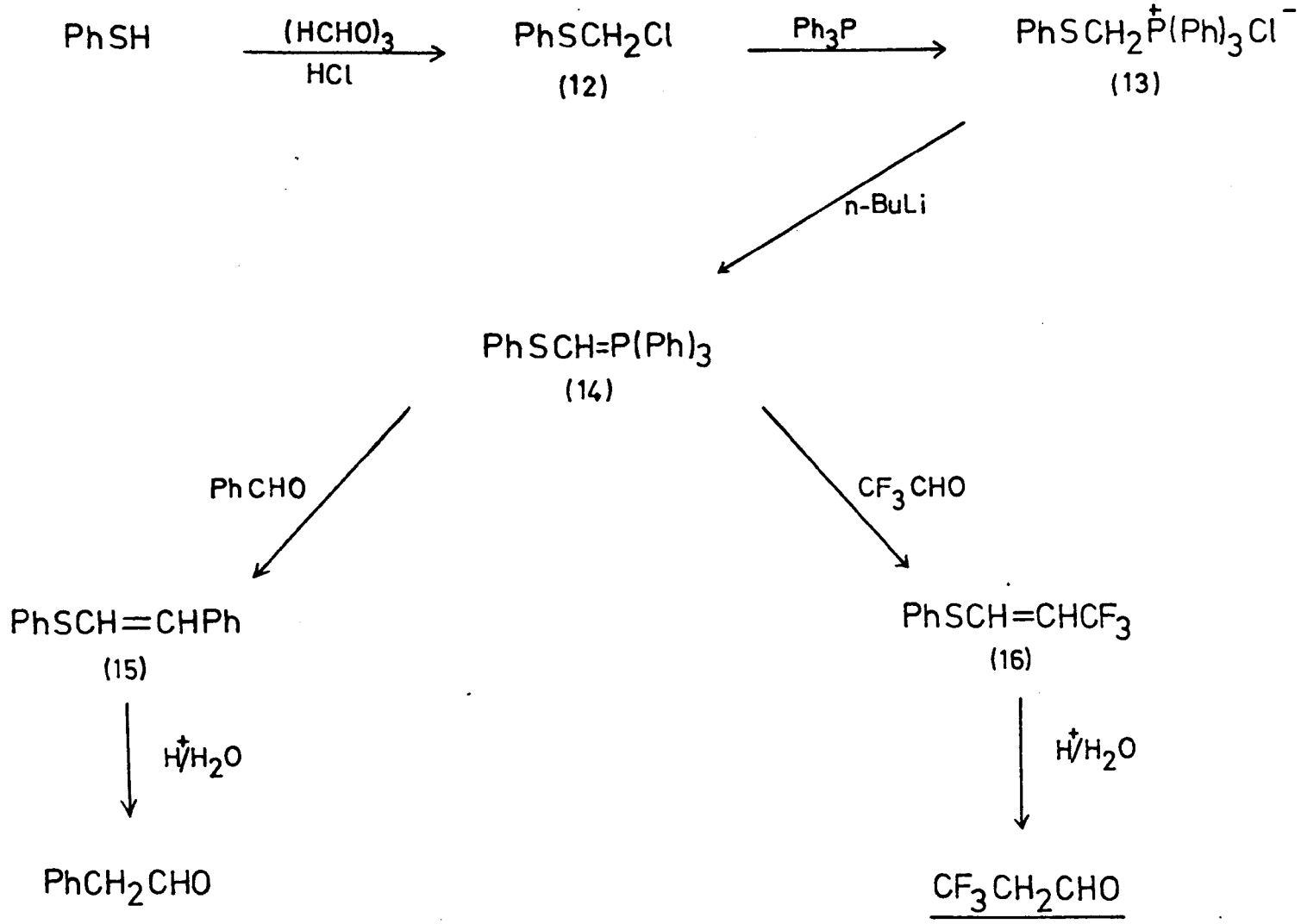
giving a mixture of compounds including unreacted triethyl orthoformate and a crystalline material. This crude material was directly hydrolysed, but the product contained very little aldehydic material. In view of the uncertainty of preparing the trifluoroethyl magnesium Grignard reagent satisfactorily, attempts to optimise this reaction were not carried out.

Via Nitriles

A possible convenient route to 3, 3, 3-trifluoropropionaldehyde is by treatment of 3, 3, 3-trifluoropropionitrile (11) with Meerwein's reagent (trialkyl-oxonium tetrafluoroborate, followed by reduction (to imine) and subsequent hydrolysis to the aldehyde product. For this route to be examined it was first necessary to synthesise trifluoropropionitrile. Trifluoropropionitrile has been prepared[154] by reacting liquid ammonia with 3-chloro-1, 1, 1-trifluoro-2-propyne at -50°C. However, this was not an attractive route, so some alternatives using more readily available starting materials were examined. Sodium cyanide in DMSO reacts[155] with alkyl halides to give the corresponding alkyl cyanide. 2, 2, 2-Trifluoroethyliodide failed to react with sodium cyanide in DMSO after prolonged reaction and heating. 2, 2, 2-Trifluoroethane-p-toluenesulphonate (10) was equally unreactive towards cyanide ion. Remarkably, after heating the tosylate in DMSO with sodium cyanide at 100°C for 30 hours, only unreacted tosylate was isolated. Refluxing gave no improvement, neither did heating the tosylate directly with sodium cyanide at 100°C for 20 hours - in both cases unreacted material was recovered.

The origin of these unusual effects lies in the CF_3 group. On the basis of inductive effects, one might expect CF_3 to enhance S_N2 displacement. However, in a study comparing the relative rates of halides in S_N2 reactions, Bordwell[156] concluded that the CF_3 group had a strong deactivating effect when attached to the α - carbon. These deactivating effects are attributed to steric and field effects, the inductive effect being assumed to be mildly rate enhancing. It was also found that[156] the reaction of 2, 2, 2-trifluoroethyl-p-toluenesulphonate with potassium iodide in acetone at 75°C, was 0.00007 times slower relative to the rate of propyl-p-toluene-sulphonate at 40°C.

These results explain the failures encountered in nucleophilic displacement reactions attempted with substituted trifluoroalkanes. The failures to synthesise 3, 3, 3-trifluoropropionitrile, and the recognised difficulties prompted an examination of some alternative schemes.


PhSH
(HCHO)3
HCl
PhSCH2Cl
(12)
Ph3P
PhSCH2P(Ph)3Cl
(13)
n-BuLi
PhSCH=P(Ph)3
(14)
PhCHO
CF3CHO
PhSCH=CHPh
(15)
PhSCH=CHCF3
(16)
H+/H2O
H+/H2O
PhCH2CHO
CF3CH2CHO

Wittig Condensations[157]

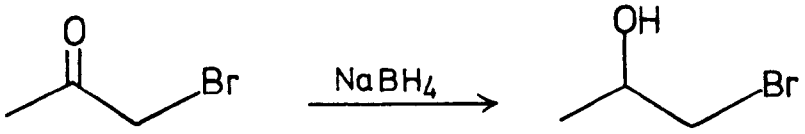
This area of study is currently still under some development. Promising results have been obtained indicating that 3, 3, 3-trifluoropropionaldehyde can be obtained by Wittig-type reactions, although some complexities have yet to be overcome.

Phenylchloromethyl thioether[158] (12) was conveniently prepared by condensing thiophenol with paraformaldehyde in the presence of hydrogen chloride. (12) reacts slowly with triphenylphosphine yielding the crystalline phosphonium salt (13). The use of this reagent for preparing homologous aldehydes was examined in a model reaction with benzaldehyde. The Wittig reagent (14) was prepared by treating the phosphonium salt (13) in DMSO with n-butyllithium.[157, 159] (14) was reacted directly with benzaldehyde to give a high yield of β-phenylmercaptostyrene (15). Some preliminary hydrolysis experiments were carried out on this material in aqueous acid. The aldehyde product was converted in situ to its 2, 4-dinitrophenyl hydrazone and comparison with authentic phenylacetaldehyde 2, 4-DNP confirmed its identity.

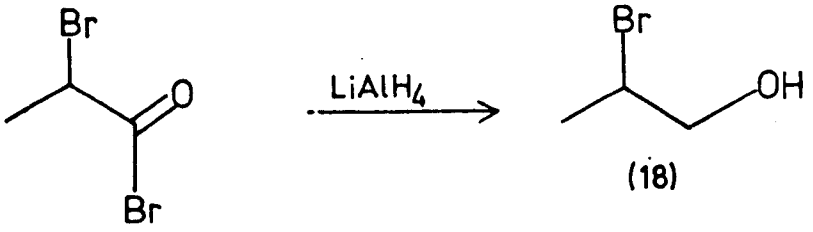
These encouraging results prompted an examination using trifluoroacetaldehyde. The Wittig reagent (14) was prepared then reacted with anhydrous trifluoroacetaldehyde. For small scale preparations of anhydrous trifluoroacetaldehyde, the dehydration of trifluoroacetaldehyde hydrate was most conveniently carried out by using phosphorus pentoxide in n-dibutyl ether. On reacting the trifluoroacetaldehyde with (14) only a low yield of phenylmercaptotrifluoropropene (15) was formed. The major conversion was instead to thioanisole.

Some preliminary hydrolysis studies were carried out on this crude material by refluxing with acidic 2, 4-dinitro phenyl hydrazine reagent for several minutes. The 2, 4-DNP derivative formed was identified as 3, 3, 3-trifluoropropionaldehyde - 2, 4-DNP by comparison with material obtained from the enzymic dehydration of trifluoropropanediol. Further work has to be carried out in order to optimise the yield of compound (16) and then define conditions for its conversion to trifluoro-propionaldehyde.

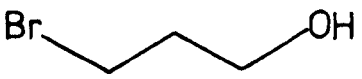
The interesting reactions observed with the glyceroldehydrase (see below) make it absolutely necessary that trifluoropropionaldehyde is made available to observe its interaction with the enzyme and buffers.



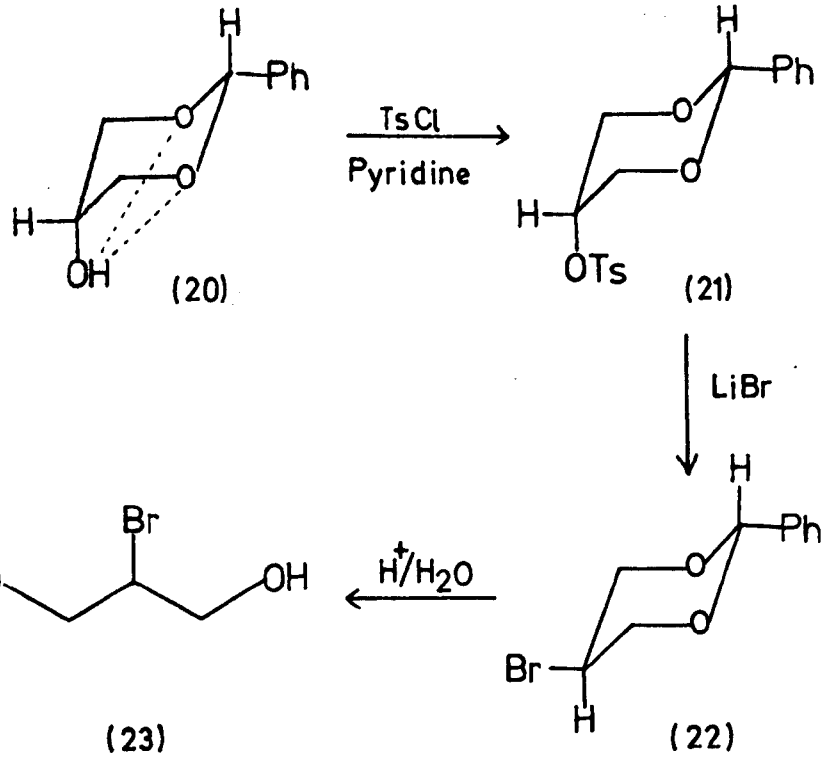
(17)



(18)



(19)



(20) (21) (22) (23)

Synthesis of other Halogenated Propanols

It was appropriate to test other halogenated propanols as substrates for glyceroldehydrase. For example with 2-bromo-1-propanol, formation of a radical at the 1-position should then lead to a 2, 1 bromine transfer. Elimination of hydrogen bromide from the geminal bromohydrin then affords propionaldehyde as the product. Likewise with 1-bromo-2-propanol, since the enzyme exhibits a preference for radical formation at the 1-position, propionaldehyde could arise via a 2, 1 hydroxyl shift. On this rationale, 2-bromo-1, 3-propanediol should be converted to 3-hydroxypropionaldehyde.

Bromopropanols

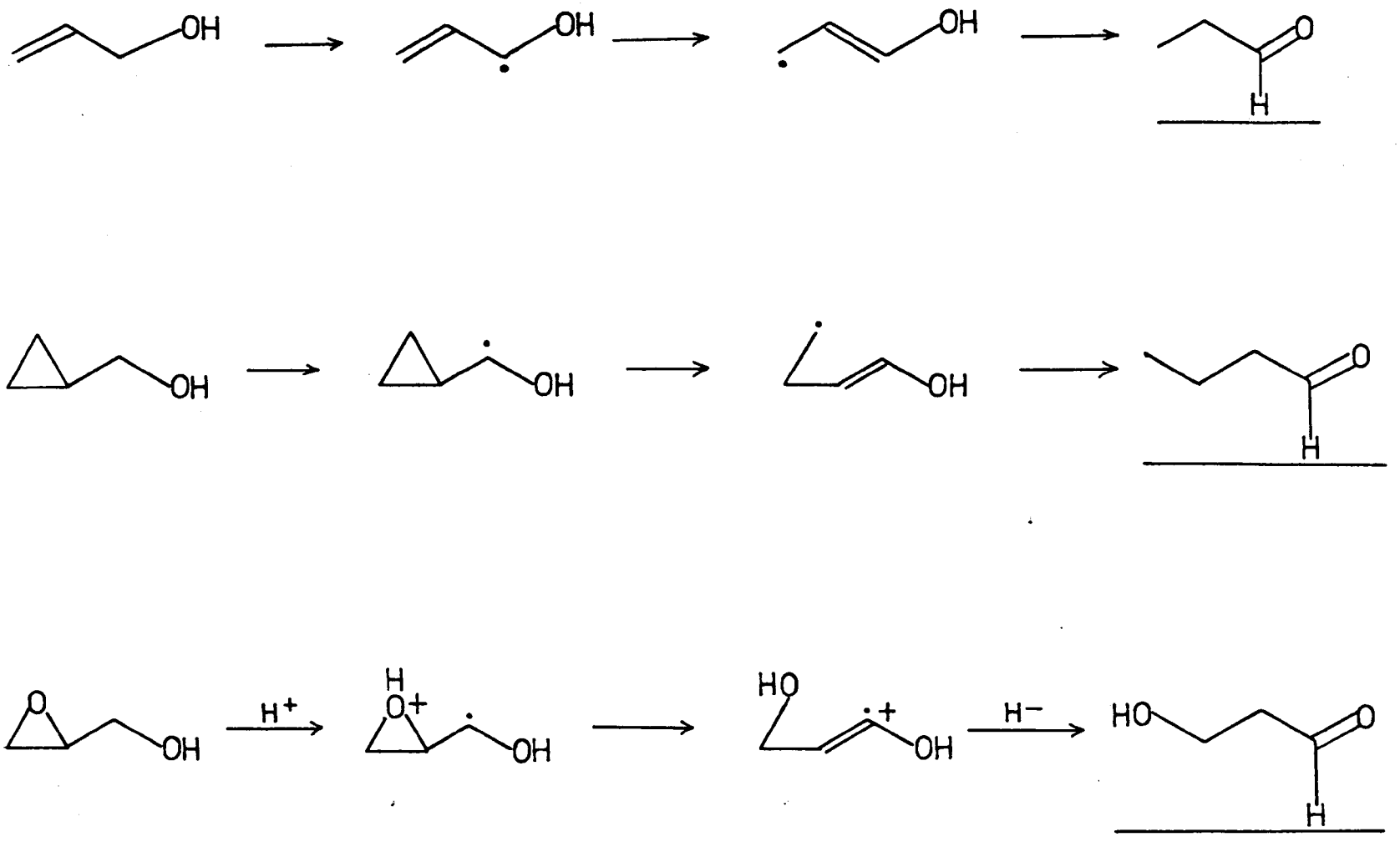
1-Bromo-2-propanol was prepared by reduction of bromoacetone with sodiumborohydride. Conditions were not found whereby lithium aluminium hydride effected a clean reduction as reported[160], without the formation of isopropanol. However, lithium aluminium hydride was satisfactory for reducing 2-bromoacetyl-bromide to 2-bromo-1-propanol. 3-Bromo-1-propanol was commercially available.

2-Bromo-1, 3-propanediol

cis-2-Phenyl-1, 3-dioxan-5-ol (20) was prepared by the condensation of glycerol and benzaldehyde, and was converted to 5-bromo-2-phenyl-1, 3-dioxan (22) via the 5-p-toluenesulphonate (21) using literature procedures.[161, 162] This latter compound could not be catalytically hydrogenolysed to the bromo-diol (23) perhaps due to poisoning of the catalyst by traces of HBr. However, the bromo-dioxan was hydrolysed to the diol, but required careful separation from the benzaldehyde also produced.

Other Possible Substrates

On the basis that allylalcohol, glycidol, and cyclopropylcarbinol might yield aldehydic products, they were tested as possible substrates. Plausible schemes whereby these could react with glyceroldehydrase are shown in Scheme I. Cyclopropylcarbinol was prepared by reduction of ethoxycarbonyl cyclopropane with lithium aluminium hydride. Allyl alcohol and glycidol were commercially available.



SCHEME I

Reactions of Glyceroldehydrase

With R, S-3, 3, 3-Trifluoro-1, 2-propanediol

Preliminary experiments[163] to test the reaction of R, S-3, 3, 3-trifluoro-1, 2-propanediol with glyceroldehydrase indicated that it is a poorly reacting substrate. Trifluoropropanediol gave ca. 55% inhibition of the enzyme activity with 1, 2-propanediol. In the initial stages of the reaction the rate did not show any non-linearity as is observed with ethanediol or glycerol. When run for longer periods (2 hrs), however, the reaction rate progressively decreased. According to assay using MBTH (N-methylbenzothiazolone hydrazone), only 44% of the trifluoropropanediol was observed as the aldehyde, so it is necessary that the MBTH assay is calibrated with synthetic 3, 3, 3-trifluoropropionaldehyde. The short term kinetics measurements give a value of 0.42 mM for the Km of trifluoropropanediol. In comparison, values for 1, 2-propanediol and glycerol are 0.07 mM and 0.57 mM respectively, suggesting that trifluoropropanediol binds quite well. Long term kinetics experiments indicated that the enzyme was completely stable for 100 minutes at 37°C in the presence of trifluoropropanediol; adding the coenzyme after this treatment causes a normal reaction. When reaction ceased it was not due to loss of coenzyme activity since adding more had no effect. Adding more trifluoropropanediol once reaction had stopped had no effect either, indicating that the falling off in the rate of reaction was not due to the exhaustion of substrate. Addition of more enzyme caused further reaction, initially as fast as in the first addition of enzyme, but the reaction ceased quickly indicating a lesser extent of reaction. The decay in reaction rate might be due to products acting as inhibitors or possibly due to the enzyme making mistakes as does occur with glycerol. Inhibitory experiments showed that acetone has no effect on the enzyme reacting with 1, 2-propanediol. Fluoride ion also did not show any effect on the enzyme.

In a preparative experiment between the enzyme containing tricine (N-tris-(hydroxymethyl)-methylglycine) and trifluoropropanediol, with ammonium phosphate buffer, the products were converted in situ to 2, 4-DNP derivatives. Separation of 2, 4-DNP derivatives confirmed the presence of 3, 3, 3-trifluoropropionaldehyde-2, 4-DNP which was identified by its characteristic n.m.r. spectrum (See Fig. 1) and its melting point. The yield of this material was only 10% of theory, suggesting that the trifluoropropanediol was not converted wholly to trifluoropropionaldehyde or the

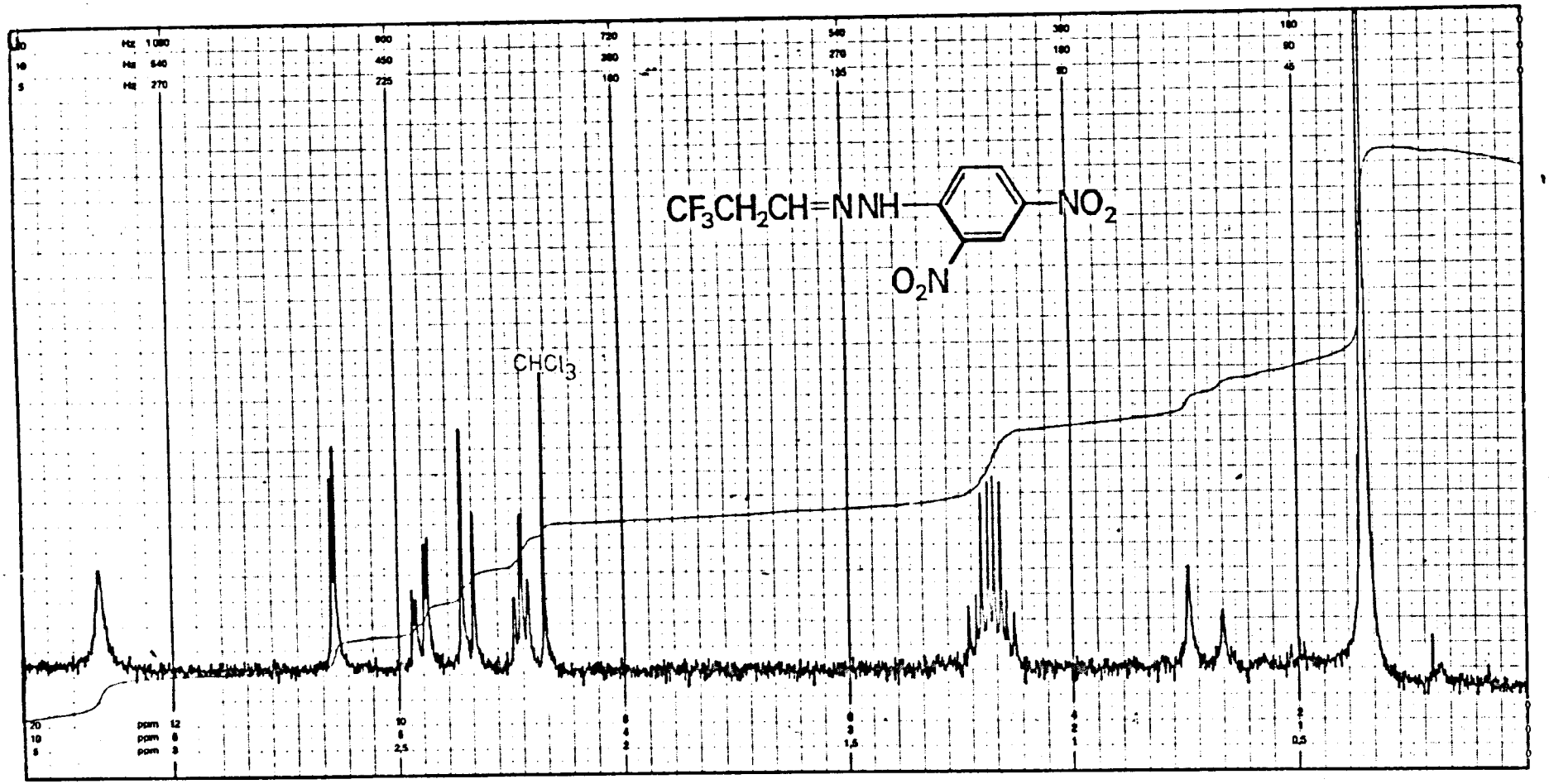


Figure 1 90 MHz ^{1}H nmr spectrum of 3, 3, 3-Trifluoropropionaldehyde-2, 4-dinitrophenylhydrazone

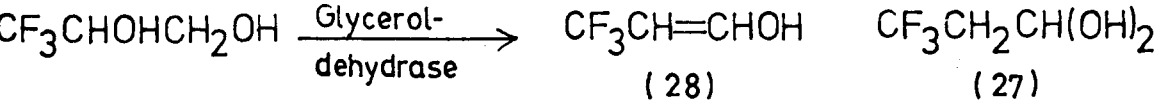
aldehyde was not passive to the reaction conditions. Also isolated was acetone-2,4-DNP which was present in greater quantity than trifluoropropionaldehyde 2,4-DNP. It is difficult to rationalise how acetone can have arisen from the enzymatic reaction. It seems unlikely that acetore could be a contaminant in the enzyme solution, although the MBTH assay would not detect it, since this method is only sensitive to aldehydes. Suitable control experiments on the reagents used confirmed that acetone could not have been introduced via this source and of course all reagents used for the enzyme reaction were purified so as to be carbonyl-free. 1,1,1-trifluoroacetone, an unlikely product, does not exchange its fluorine atoms for hydrogens and significant amounts of acetone-2,4-DNP were not isolated from control reactions on 1,1,1-trifluoroacetone-2,4-DNP. The source of the acetone is still a mystery although repeat reactions have not been carried out due to the complexities revealed by the ^{19}F n.m.r. studies.

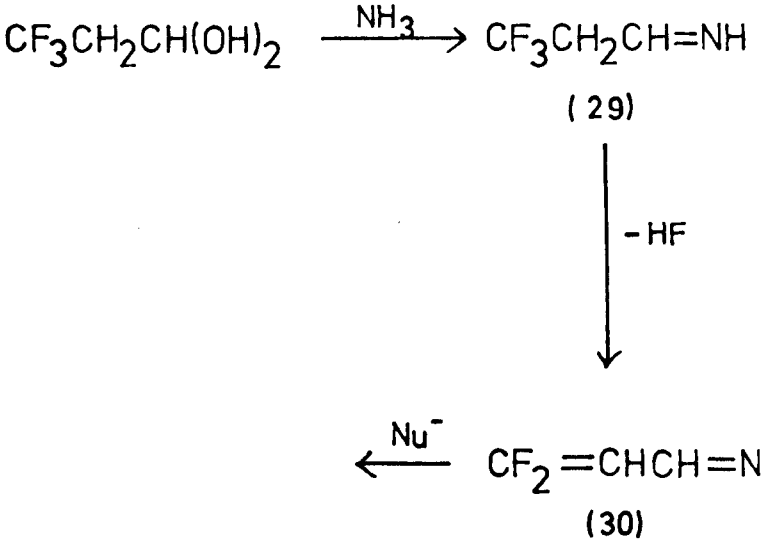
Nuclear Magnetic Resonance Observations

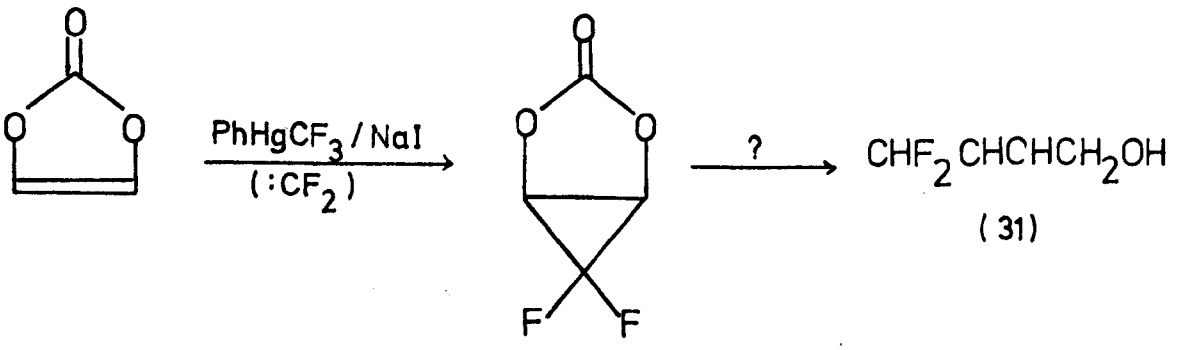
Preliminary studies[164] on the reaction of trifluoropropanediol with glycerol-dehydrase, using ^{1}H n.m.r. spectroscopy, have failed to reveal the production of acetone during the reaction. The enzyme used for these experiments contained tricine which complicated the spectra, since its signals overlapped those of the trifluoropropanediol. ^{1}H n.m.r. studies using tricine-free enzyme have to be carried out.

^{19}F N.m.r. Observations

Monitoring the enzymatic reactions of trifluoropropanediol with glycerol-dehydrase by ^{19}F n.m.r. indicates why a low conversion of trifluoropropanediol to trifluoropropionaldehyde was observed in the preparative experiment. In the majority of reactions examined by ^{19}F n.m.r., fluoride ion was a product being observed as a singlet at δ - 124.7 (relative to $CFCl_3$). It was identified by the addition of sodium fluoride. The proportion of fluoride ion produced depends critically on the conditions employed for the reaction. Experiments using different preparations of enzyme indicated that tricine had no effect on the distribution of products. The effects of buffer and pH, however, are more important. Using ammonium phosphate as buffer, fluoride ion is a major product. With potassium phosphate, fluoride ion production is very low. The effect of ammonia on the products of the reaction has been convincingly demonstrated by addition of ammonium


CF3CHOHCH2OH
Glycerol-
dehydrase
CF3CH=CHOH
(28)
CF3CH2CH(OH)2
(27)


CF3CH2CH(OH)2
NH3
CF3CH2CH=NH
(29)
-HF
Nu-
CF2=CHCH=NH
(30)


PhHgCF3 / NaI
(:CF2)
?
CHF2CHCHCH2OH
(31)
F
F

sulphate to a reaction previously buffered and run with potassium hydrogen phosphate at pH 8.0. Initially, the doublet at δ - 79.4 (J = 12Hz) for the trifluoropropanediol diminished, forming a triplet at lower field (δ – 65.3, J = 12Hz) assigned to trifluoropropionaldehyde; very little fluoride ion was produced. The addition of ammonium sulphate caused a dramatic increase in the intensity of the signal at δ - 124.7 due to fluoride ion, with simultaneous diminution of the low field triplet. Interestingly, addition of ammonium sulphate to a similar reaction run at pH 6.5 had no effect. The assignment of the triplet at δ - 65.3 to trifluoropropionaldehyde has yet to be confirmed, making the synthesis of authentic material imperative, since it is also necessary to examine a control non-enzymatic reaction between 3, 3, 3-trifluoropropionaldehyde and ammonium buffers. The structure of the product from the enzymatic reaction is still uncertain. In aqueous solution it is highly probable that the aldehyde is hydrated. It is surprising that the removal of the hydroxyl group at C - 2 in 3, 3, 3-trifluoropropanediol and subsequent replacement at C - 1, should cause a downfield shift on the CF_3 fluorine atoms. The aldehyde is certainly not present as the enol form (28) as the CF_3 group would be seen as a doublet.

It is possible that the elimination of fluoride ion from the product is enzyme controlled, although experiments indicated that all the diol is consumed with no product inhibition. A more likely possibility is simply that ammonia reacts with 3, 3, 3-trifluoropropionaldehyde to give the imine (29) which, in the presence of base, eliminates HF, giving (30). Further nucleophilic attack by ammonia or water may expel the remaining fluorine atoms. The above-mentioned controls should confirm this hypothesis when authentic trifluoropropionaldehyde is available.

A further complication with some of the enzymatic reactions is the appearance of another product signal at δ - 93.3, also a triplet, although its shape changed with time, becoming more like a doublet. This product may be the enol form of trifluoropropionaldehyde, which would account for the doublet splitting pattern. However, it does seem highly unlikely that this species could exist in an aqueous environment. Also the chemical shift for a CF_3 group attached to an olefinic carbon atom is usually in the range -55 to -75 ppm[165] to the high field of $CFCl_3$. This product was observed using several enzyme preparations, with or without tricine. This signal only formed when using potassium phosphate buffers and no fluoride ion

formed when it appeared. The effect of ammonium salts on this product has not been examined. It is, of course, possible that this signal is due to trifluoropropionaldehyde and that the earlier arguments for the assignment of the low field triplet at δ -65.3 are not correct. This is a further reason for requiring an authentic specimen of trifluoropropionaldehyde

In conclusion, it appears that the enzymatic dehydration of trifluoropropanediol is complex, with the cation perhaps playing a part in the reaction pathway. It is uncertain whether the effect of the cation is controlled by the enzyme. The presence of the CF_3 group ought to stabilise a radical formed at C-2 of 3, 3, 3-trifluoropropanediol, but since 3, 3, 3-trifluoropropionaldehyde is definitely a product this suggests that the initial radical forming step is at C-1, the primary alcohol.

Although the magnetic resonance studies have yet to reveal the nature of intermediates in the dioldehydration, ^{19}F n.m.r. spectroscopy holds distinct advantages for in situ observation in a predominantly protic environment. Several complexities have been revealed when using fluorinated substrates - it is also possible that the natural substrates undergo complex transformations which have been hitherto unobserved. With the natural substrates, only the 2, 4-DNP derivatives of products have been isolated and the study of intermediates by magnetic resonance methods has been restricted to e.s.r.

Future Work with Fluorinated Substrates

The interesting observations made concerning the reactions of 3, 3, 3-trifluoro-1, 2-propanediol suggest that other fluorinated substrates are worthy of some investigation. We propose to synthesise 3, 3-difluoro-1, 2-propanediol (31) via the attack of difluorocarbene on vinylene carbonate. The ring system is easily opened by alkali and should afford a convenient route to the diol. The reactions of difluoropropanediol with glyceroldehydrase will certainly be worth examining since Richards[166] has found that propanedioldehydrase converts R, S-3-fluoro-1, 2-propanediol specifically to 3-hydroxypropanal rather than 3-fluoropropanal. The reaction proceeds at about 25% of the rate for natural substrates.

It is probable that 3, 3-difluoro-1, 2-propanediol will also be a substrate and may undergo interesting fluorine elimination reactions.

Reactions of Other Potential Substrates

Bromopropanols

1-Bromo-2-propanol showed only weak inhibition of glyceroldehydrase when tested against 1,2-propanediol, and it is not an effective substrate. 2-Bromo-1-propanol is also a very weak inhibitor but not an active substrate, reacting at less than 0.1% of the rate of 1,2-propanediol. 3-Bromo-1-propanol is not an active substrate either, and is only a weak inhibitor. The preliminary results[163] suggest that 3-bromo-1-propanol is a simple competitive inhibitor for reversible binding, with propanediol. Further experiments were not carried out since the inhibition caused by these bromopropanols was so weak.

2-Bromo-1,3-propanediol

2-Bromo-1,3-propanediol inhibits the reaction between the enzyme and 1,2-propanediol. The per cent inhibition varies with the concentration of 2-bromo-1,3-propanediol indicating that the effect is not simple competition. High concentrations of 1,2-propanediol reduce the inhibiting effect. 2-Bromo-1,3-propanediol is a much stronger inhibitor than 2-bromo-1-propanol, suggesting that a primary alcohol at C-3 gives better binding that when this is replaced by a CH_3 group. Also 2-Bromo-1,3-propanediol probably competes with 1,2-propanediol and then undergoes an irreversible step when the coenzyme is present.

Allyl Alcohol

Allyl alcohol is not a substrate, but causes about 50% inhibition when in a ratio of 10 : 1 to 1,2-propanediol. The inhibition decreases as the concentration of 1,2-propanediol is increased. Preliminary competitive studies suggest that allyl alcohol competes with 1,2-propanediol for binding then undergoes an irreversible reaction with the enzyme.

Cyclopropylcarbinol

Cyclopropylcarbinol was neither a substrate nor an inhibitor for glyceroldehydrase.

Glycidol

The reactions of glycidol are somewhat more complex, although it appears that glycidol does act as a poor substrate. The results are complicated by the fact that the zero-time control assay for aldehyde is much higher using MBTH than

2, 4-DNP reagent. The 'blanks' also increased with the age of the glycidol. It is possible that glycidol reacts with these test reagents and control experiments have to be carried out before any definite conclusions can be made. Preliminary measurements indicate that the extent of the reaction is complete within 5 minutes, showing very similar rate characteristics to glycerol. It is, of course, possible that glycidol undergoes enzyme catalysed hydration to glycerol; hydration in an alternative rapid manner is unlikely, since reactions were buffered at pH 7.8. Before more accurate conclusions can be made concerning these reactions, it is necessary that some reactions of glycidol under defined chemical conditions are examined, to be sure that glycidol is not transformed into a substrate prior to its interaction with the enzyme. It is anticipated that these studies will be carried out in the near future.

EXPERIMENTAL V

Fluoral cyanohydrin (3)

2,2,2-Trifluoroacetaldehyde hydrate (2) was commercially available and was redistilled (b.p.98°C) before use. 2,2,2-Trifluoroacetaldehyde hydrate (6.9g, 65mM) was added dropwise during 12 mins to a solution of NaCN (3.34 g of 94%, 65.3 mM) in water (12 ml) in an ice-salt bath maintaining the temperature at -5 - 0°C. 6 N H_2SO_4 (24ml) was carefully added to the solution, keeping the temperature below 2°C. After 45 minutes the suspension became very viscous, although after 70 minutes the viscosity decreased. Addition was complete in 90 minutes, after which the orange suspension was allowed to warm to room temperature and stirred for 4 hours. Nitrogen was bubbled through the orange solution for 75 minutes to expel HCN. The solution was extracted with Et_2O (3 x 20 ml), and extracts were washed with saturated brine (20 ml) and dried. Evaporation yielded a brown oil 6.19 g (76% crude yield) which was stored at -20°C if not distilled immediately. Distillation in vacuo gave a colourless oil (4.72 g, 58%) b.p.80.7 - 82.5°C/40 mm (lit.[149] bp 59 - 60°C/17 mm).

i.r. (liq. film) : ν cm^{-1} 3400 br s, 2940 m, 1426 m, 1345 s, 1267 s, 1206 s, 1154 s, 1108 s, 1042 w, 954 s, 840 s, 715 s.

n_D^{18} : 1.3344 (lit.[149] n_D^{20} 1.3330)

3,3,3-Trifluorolactic Acid (4)

To avoid loss of the cyanohydrin by polymerisation, only small scale experiments were performed.

Fluoral cyanohydrin (4.70 g, 37.6 mM) was cautiously added dropwise to conc. H_2SO_4 (4.7 ml) during ca.5 mins, resulting in a thick white paste. The temperature was raised to 120°C for 15 mins, then the mixture was cooled. Iced water (25 ml) was cautiously added, giving a colourless solution which was refluxed for 15 hours. The above procedure was repeated on an identical scale and the products combined. The aqueous solution was saturated with anhydrous Na_2SO_4 then extracted with Et_2O (4 x 30 ml). Extracts were washed with saturated Na_2SO_4 (20 ml) then dried (Na_2SO_4). Evaporation gave a colourless viscous oil (10.0 g, 92%) which was distilled in vacuo b.p.57 - 57.5°/1 mm (lit.[147] b.p.60 - 95°/1 - 2 mm). On distillation, the product crystallised immediately, and it was necessary to extract some product from the condenser of the distillation apparatus (total 8.7 g,

80%). The trifluorolactic acid was used directly.

m.p. : 64 - 67°C (lit.[147] 68 - 69°C)

n.m.r. ($CDCl_3/CD_3OD$): δ = 4.55 (q, 7, 1H), 6.45 (s, 2H)

i.r. (CH_2Cl_2) : νcm^{-1} 3500 br ms, 2920 w, 2850 w, 1765 s, 1739s

Ethyl 3, 3, 3-trifluorolactate (5)

1-Ethyl-3-p-toluene triazene was recrystallised from pentane before use. 3, 3, 3-trifluorolactic acid (7.2 g, 50 mM) in Et_2O (50 ml) was added during 15 mins to a solution of 1-ethyl-3-p-toluene triazene (11.4 g, 70 mM) in Et_2O (100 ml). Effervescence began almost immediately, and ceased ca. 15 mins after addition was complete. The orange solution was stirred a further 1 hour at room temperature, then washed with 2N HCl (50 ml), followed by 5% aq. $NaHCO_3$ (50 ml). The etheral solution was dried (Na_2SO_4), then decolourised with activated charcoal. Careful evaporation yielded almost colourless crystals (6.83, 78%) which yellowed on standing. (N.B. These crystals were extremely volatile). This material was directly reduced to the diol.

In a small scale experiment the product was purified by sublimation (80°C/70 mm) onto a cold finger (-78°C) then recrystallised from pentane.

m.p. : 36.5 - 37°C

n.m.r. (CCl_4) 100Mhz : δ = 1.22 (t, 7, 3H), 3.46 (s, 1H), 4.30 (m, 3H)

i.r. (CH_2Cl_2) : νcm^{-1} 3400 m, 2980 m, 2940 m, 2910 m, 1745s

		% C	H	F
$C_3H_7O_3F_3$	requires:	34.85	4.10	33.14
	found:	34.66	4.00	32.88

R, S- 3, 3, 3-Trifluoro-1, 2-propanediol (1)

Ethyl 3, 3, 3-trifluorolactate (6.8 g, 40 mM) in dry Et_2O (25 ml) was added dropwise during 15 mins to lithium aluminium hydride (1.9 g, 50 mM) in dry Et_2O (50 ml) maintaining gentle reflux. The suspension was stirred for a further $3\frac{1}{2}$ hrs at room temperature, before the excess lithium aluminium hydride was destroyed by careful addition of water (6 ml). The suspension was stirred for 1 hr, saturated with Na_2SO_4 and then stirred with Et_2O (50 ml). The suspension was centrifuged and the Et_2O layer separated. The residue was extracted with Et_2O (3 x 50 ml) as above, centrifuging each time. Etheral extracts were combined; drying and

evaporation yielded a brown oil (2.3 g). The aqueous residue was acidified to ca. pH 2 with 2N H_2SO_4, then continuously extracted with Et_2O (300 ml) for 18 hours, to give a further 2.2 g of a milky oil. The products were combined, dissolved in dry Et_2O, centrifuged then filtered through Celite. Evaporation gave a brown oil (4.4 g, crude yield 84%). (N.B. Traces of inorganic material were difficult to remove and in general suspensions were centrifuged. Solutions were only filtered when almost perfectly clear). The crude product was distilled in vacuo although some difficulty with foaming was encountered, so a freeze-thaw technique was used to degas the material prior to distillation. The 3, 3, 3-trifluoro-1, 2-propanediol distilled as a pale yellow oil (b.p. 93 - 94°C/27 mm) (lit.[146] 69.5 - 70.1°/10 mm) (1.68 g, 32%) although a high proportion of the crude material polymerised during the distillation.

For the enzymatic studies, the diol was distilled satisfactorily a further two times in vacuo (b.p. 99.5 - 100°C/35 mm). The material solidified on cooling.

n.m.r. ($CDCl_3/CD_3OD$): δ = 3.55 - 4.25 (m, including 3.8 (s))

i.r. (liq. film) : νcm^{-1} 3360 br s, 2950 mw, 2900 mw, 1276 s, 1179 s, 1130 s, 1170 m, 1135 m, 984 w, 908 w, 860 w, 752 w, 706 m, 670 m

n_D^{18} : 1.362 (lit.[146] n_D^{20} 1.3617)

	% C	H	F
Calc for $C_3H_5F_3O_2$:	27.70	3.87	43.82
found:	27.68	3.85	42.45

The α-naphthylurethane derivative was prepared according to Shriner[167] using dry pyridine as catalyst and was obtained as a white solid which was recrystallised from $CHCl_3/CCl_4$ to yield white crystals m.p. 201 - 202°C (lit.[146] m.p. 198 - 199°C).

Attempted Preparation of 3, 3, 3-Trifluoropropionaldehyde

Reaction of 2, 2, 2-trifluoroethyliodide with lithiumdithiane[87a, 151]

1, 3-Dithiane (600 mg, 5 mM) was dissolved in dry THF (5 ml) under N_2 and cooled to -5°C. n-Butyl lithium (2.6 ml of a 2.1 M solution in hexane, 5.5 mM) was added dropwise, maintaining the temperature below 5°C. Stirring was continued for 30 mins at -5 - 0°C then 2, 2, 2-trifluoroethyl iodide (1.05 g, 5 mM) was added,

keeping the temperature at ca. 0°C. The first few drops of the iodide caused an exotherm but further additions produced no temperature increase. The solution was stirred at -5°C for 1 hour then allowed to warm to room temperature and stirring was continued for 22 hours. The solution was still basic, so 1N HCl (0.5 ml) was added to neutralise, and then the products were extracted with CH_2Cl_2 Drying and evaporation gave a brown oil (700 mg) which was many spots by t.l.c. The n.m.r. spectrum confirmed that this material was predominantly 1,3-dithiane. In a similar experiment run for a shorter period, 1, 3-dithiane was recovered quantitatively and its identity was confirmed by comparison of its spectroscopic properties and mixed melting point with authentic material.

Preparation of 2, 2, 2-Trifluoroethyl-p-Toluene Sulphonate

2, 2, 2-Trifluoroethyl-p-toluene sulphonate (10) was prepared from 2, 2, 2-trifluoroethanol and p-toluene sulphonyl chloride in pyridine according to Edgell.[168] It was recrystallised from petrol (40 - 60°).

m.p. : 40°C (lit.[168] 41°C)

n.m.r.($CDCl_3$) : δ = 2.45 (s, 3H), 4.36 (q, 8, 2H), 7.40 (d, 9, 2H) 7.85 (d, 9, 2H).

Reaction of 2, 2, 2-Trifluoroethyl-p-Toluene Sulphonate with Sodium Cyanide

In DMSO

2, 2, 2-Trifluoroethyl-p-toluene sulphonate (2.86 g, 10 mM) in dry DMSO (2 ml) was added to a slurry of NaCN (590 mg, 12 mM) in DMSO (3 ml) at 50°C. There was no exotherm but the solution turned orange and was allowed to cool to room temperature. After 3 hours, no reaction had occurred, so the solution was heated to 100°C. The mixture was dark brown after heating for 30 hours but t.l.c. showed no new products.

An identical reaction was carried out heating the mixture to reflux in DMSO for 1 hour. The mixture was then placed in a distillation apparatus and heated at atmospheric pressure, but no 3, 3, 3-trifluoropropionitrile (b.p. 92°) distilled out.

Direct Fusion

2, 2, 2-Trifluoroethyl-p-toluene sulphonate (286 mg, 1 mM) and NaCN (59 mg, 1.2 mM) were heated at 100°C under argon in a flask fitted with a reflux condenser. After 20 hours only starting material could be identified.

Reaction of 2, 2, 2-Trifluoroethyliodide with Sodium Cyanide

2, 2, 2-Trifluoroethyliodide (1.05 g, 5 mM) was added dropwise to a slurry of NaCN (590 mg, 12 mM) in dry DMSO (4 ml) at 50°C, in a reaction vessel fitted with a CO_2/acetone condenser to prevent loss of any volatile products. A slight exotherm to 60°C occurred and the orange suspension became very viscous after 1 hour. After 2 hours the mixture was cooled to room temperature, then diluted with water and extracted well with CH_2Cl_2. The purple extracts were washed with water and the purple colour transferred to the aqueous phase. Drying and careful distillation of the organic extracts at atmospheric pressure gave no 3, 3, 3-trifluoropropionitrile.

Phenylchloromethylthioether (12)

Phenylchloromethylthioether was prepared by bubbling anhydrous HCl through a mixture of paraformaldehyde and thiophenol maintained at -15° to -5°C as described by Böhme.[158] The product was a colourless oil (b.p. 79° - 85°C/2.5 mm, lit.[158] 98°/12 mm) (36%).

n.m.r.($CDCl_3$) : δ = 4.92 (s, 2H), 7.20 - 7.70 (m, 5H).

Phenylmercaptomethyl-triphenyl phosphonium chloride (13)

Triphenylphosphine (26.2 g, 0.1 M) and phenylchloromethylthioether (15.85 g, 0.1 M) were dissolved in dry benzene (25 ml) and heated to reflux. After 14 hours the crystals formed were filtered off and washed with cold benzene. Drying gave white crystals (25.6 g, 61%) which were one component by t.l.c. The filtrate was refluxed for a further 48 hours after which a second crop of crystals (9.5 g) were obtained (total yield 83%).

n.m.r.($CDCl_3$) : δ = 5.60 (d, 2H), 6.98 - 8.20 (m, 20H).

Dehydration of 3, 3, 3-Trifluoroacetaldehyde hydrate[169, 170]

Trifluoroacetaldehyde hydrate (5.8 g, 25 mM) was added to a slurry of P_2O_5 (20 g) in n-Bu_2O (50 ml) at 100°C. The resulting gaseous trifluoroacetaldehyde was passed through glass wool (previously dried at 110°C for 24 hours) at 0°C and then condensed in a calibrated trap at -78°C (CO_2/acetone). When the dehydration had ceased ca. 4 ml (at -78°C) of product had collected. The yield of the pure aldehyde was variable, and it appeared that some polymerised material may have formed on the glass wool. Conditions for maximum yield have yet to be optimised. The aldehyde was purified by trap to trap distillation.

Preparation of Phenylmercapto-3, 3, 3-trifluoro-2-propene (16)

Phenylmercaptomethyl-triphenylphosphonium chloride (13) (18.9 g, 45 mM) in dry DMSO (100 ml) was cooled to 10 - 15°C under argon. n-Butyl lithium (21.4 ml of a 2.1 M solution in hexane, 45 mM) was added dropwise during 10 minutes maintaining the temperature at 15 - 25°C. A yellow solution initially formed, but soon became dark orange. Stirring was continued for 15 mins at 20°C after addition was complete. 2, 2, 2-Trifluoroacetaldehyde (ca. 3.2 ml assumed d ≃ 1.2, 40 mM) was allowed to vaporise into the Wittig reagent via a stainless steeltube. The addition required 10 mins and there was no exotherm. Stirring was continued for 1 hour after which time no starting materials were left. The reaction mixture was poured on to crushed ice and then extracted with Et_2O. Extracts were washed with brine then water. Drying and evaporation yielded a brown crystalline solid (16.5 g) which was chromatographed on silica gel (1 : 1 CH_2Cl_2/petrol (60 - 80°)). The first fractions contained the desired product but were a mixture of three components (total 5.2 g) by g.l.c. (6' 20% DEGS, 120°C). The mixture consisted of predominantly one component (ca. 70%) which was identified as thioanisole by comparison with authentic material (equivalent to ca. 65% conversion).

n.m.r.($CDCl_3$) : δ = 2.47 (s), 7.25 (m)

i.r.(liq. film) : νcm^{-1} 3060 m, 2920 m, 2850 w, 1585, 1480, 1442

This crude material was tested for phenylmercapto-3, 3, 3-trifluoro-2-propene by refluxing with 2, 4-dinitrophenyl hydrazine reagent in phosphoric acid for several minutes. The yellow precipitate was extracted with CH_2Cl_2 and 3, 3, 3-trifluoro-proionaldehyde-2, 4 DNP was confirmed by comparison with authentic material on t.l.c. (silica gel; benzene).

2-Bromo-1, 3-propanediol
cis-2-phenyl-1, 3-dioxan-5-yl-p-toluenesulphonate

p-Toluenesulphonyl chloride (9.55 g, 50 mM) in pyridine (10 ml) at 0°C was added with stirring during 20 mins to cis-2-phenyl-1, 3-dioxan-5-ol-(9.0 g, 50 mM) in pyridine (20 ml) at 0°C. Stirring was continued for 6 hours at room temperature, then water (150 ml) was added. The pyridine hydrochloride dissolved and the tosylate precipitated out. The crude product was filtered off, then crystallised by dissolving in the minimum CH_2Cl_2, diluting with Et_2O followed by cooling which yielded a first crop of pure cis-2-phenyl-1, 3-dioxan-5 yl-p-toluenesulphonate (13.7 g, 87%).

m.p. : 125°C (lit.[171] 125°C)

n.m.r.($CDCl_3$) : δ = 2.42 (s, 3H), 4.48 (m, 1H), 5.50 (s, 1H), 7.20 - 7.70 (m, 7H), 7.90 (d, 7, 2H).

trans-5-Bromo-2-phenyl-1,3-dioxan

Pure trans-5-bromo-2-phenyl-1,3-dioxan was prepared by refluxing cis-2-phenyl-1,3-dioxan-5-yl-p-toluenesulphonate with anhydrous lithium bromide in dry acetonitrile as described by Aneja.[161] The product was recrystallised from MeOH.

m.p. : 82 - 85°C (lit.[161] 83 - 86°C)

n.m.r.($CDCl_3$) : δ = 3.71 - 4.65 (m, 5H), 5.50 (s, 1H), 7.42 (m, 5H)

Hydrolysis of trans-5-bromo-2-phenyl-1,3-dioxan

35% aqueous HCl (25 ml) was added to a suspension of trans-5-bromo-2-phenyl-1,3-dioxan (3.2 g, 13.2 mM) in MeOH (30 ml) and water (20 ml). The mixture was warmed to reflux and after 30 mins no starting material was present. Methanol was removed in vacuo and the aqueous mixture saturated with Na_2SO_4 then extracted with CH_2Cl_2 to yield an oil which contained mainly benzaldehyde. The aqueous solution was continuously extracted with Et_2O for 12 hours to yield a pale yellow oil (1.9 g), which was distilled in vacuo (b.p. 145 - 145°/25 mm; lit.[172] 136 - 138°/21 mm) to give 2-bromo-1,3-propanediol (1.34 g, 66%) which was pure by n.m.r.

For the enzymatic studies, this material was distilled in vacuo a further two times collecting mid fractions.

n.m.r. ($CDCl_3/CD_3OD$): δ = 3.80 - 4.12 (m, 5H), 4.20 (s, 2H)

i.r.(liq.film) : νcm^{-1} 3440 br s, 2940 m, 2880 m

n_D^{26} : 1.519

2-Bromo-1-propanol

Freshly distilled 2-bromoacetyl bromide (b.p. 57 - 59°C/15 mm) (12.96 g, 60 mM) in dry Et_2O (100 ml) was added cautiously during 45 mins to lithium aluminium hydride (2.28 g, 60 mM) in dry Et_2O (50 ml) cooled in ice. Stirring was continued at 0°C for 1 hour, then water (10 ml) was added dropwise to destroy the excess lithium aluminium hydride, followed by addition of 1% H_2SO_4 (50 ml). The suspension was stirred for 1 hour at room temperature, then filtered. The residue was washed with Et_2O and the aqueous filtrate extracted with Et_2O. Etheral solutions were

combined, dried and evaporated at atmospheric pressure. The residual colourless oil was distilled in vacuo (b.p. 53 - 56°C/18 mm) to give pure 2-bromo-1-propanol (lit.[173] b.p. 62.8 - 64°C/24 mm) (5.84 g, 69%). This material was distilled in vacuo twice more (b.p. 61°C/20 mm) for the enzymatic studies.

n.m.r.($CDCl_3$) : δ = 1.73 (d, 7, 3H), 2.95 (s, 1H), 3.75 (d, 7, 2H), 4.00 - 4.50 (m, 1H)

i.r.(liq.film) : νcm^{-1} 3450 br s, 2975 m, 2930 m, 2875 w

n_D^{26} : 1.478, lit.[173] n_D^{30} 1.4785

1-Bromo-2-propanol

Sodium borohydride (1.0 g, 26 mM) was added portionwise to a solution of bromoacetone (10.3 g, 75 mM) in EtOH (10 ml) and aqueous KH_2PO_4/Na_2HPO_4 buffer (pH 7.2, 10 ml) cooled in ice. The mixture was stirred 30 mins at 0°C, then 1N HCl added to ca. pH 6. The solution was saturated with anhydrous Na_2SO_4 and extracted with ether. Extracts were dried and the solvents distilled off at atmospheric pressure. The residual oil was distilled in vacuo (b.p. 50 - 54°/20 mm) to yield pure 1-bromo-2-propanol (lit.[174] b.p. 60°C/28 mm) (4.4 g, 43%) which was distilled a further two times in vacuo (b.p. 54.5°C/20 mm).

n.m.r.(pure liq): δ = 1.25 (d, 7, 3H), 3.40 (d, 7, 3H), 3.96 (m, 1H), 4.50 (s, 1H)

1-Bromo-3-propanol

1-Bromo-3-propanol was commercially available and was redistilled in vacuo (80 - 85°C/20 mm). This material was twice distilled (b.p. 84°/20 mm).

n.m.r.(pure liq): δ = 2.05 (quintet, 7, 2H), 3.50 (t, 7, 2H), 3.72(t, 7, 2H) 6.55 (s, 1H)

Cyclopropylcarbinol

Ethoxycarbonyl cyclopropane (4.6 g, 43.4 mM) in dry Et_2O (10 ml) was added dropwise during 15 minutes to lithium aluminium hydride (1.9 g, 50 mM) in dry Et_2O (50 ml). The suspension was stirred 3 hours, then cooled, and water (6 ml) followed by Et_2O (75 ml) was added, stirred for 30 mins, then filtered. The residue was washed with Et_2O, and combined etheral extracts were washed with saturated Na_2SO_4, then dried. Et_2O was removed at atmospheric pressure and the resulting colourless liquid was distilled, collecting the fraction 115 - 118°C (752 mm) (1.34 g, 43%). This material was twice redistilled.

Allyl Alcohol

Allyl alcohol was commercially available and purified by passing down an alumina column, then distilled from $CaSO_4$. This material was redistilled, b. p. 96 - 97° (755 mm).

Glycidol

Glycidol was commercially available and was doubly distilled in vacuo (71°C/14 mm).

Enzyme Reactions

The enzyme used was glyceroldehydrase extracted from Aerobacter Aerogenes PZH (Warsaw) and was purified from induced cells using 1, 2-propanediol as protectant and as a substrate for the assay of the enzyme activity. The purification of the enzyme used a method devised by Davies, Foster and Young and is described in reference 175. The final solution was in 100 mM tricine buffer (pH 8.2, 0°) and contained an undetermined amount of $(NH_4)_2\ SO_4$. Using1, 2-propanediol as substrate, two routine assays were used based on the conversion to propionaldehyde. The propionaldehyde produced was measured colorimetrically as its 2, 4-dinitrophenylhydrazone essentially as the method described by Abeles.[176] For a more sensitive assay, the propionaldehyde was measured colorimetrically by reaction with N-methylbenzothiazalone Hydrazone (MBTH), according to a modification of the method of Paz et al.[177] One unit of enzyme activity was defined as that amount which produced 1 μ M of propionaldehyde per min using the 2, 4-DNP assay under standard conditions.

Preparative Conversion of R, S-3, 3, 3-Trifluoropropanediol to 3, 3, 3-Trifluoropropionaldehyde

Analytical reagent grade 2, 4-dinitrophenylhydrazine was recrystallised from EtOH. All solvents were redistilled from 2, 4-dinitrophenylhydrazine before use. R, S-3, 3, 3-Trifluoro-1, 2-propanediol (0.4 ml of a 1 M solution in H_2O, 400 μ M), H_2O (25 ml), and ammonium phosphate (1.5 ml of a 0.5 M solution, pH 7.8) were mixed with glyceroldehydrase (0.2 ml of a solution 540 units/ml; 6.4 mg/ml in 100 mM tricine pH 8.2). The solution was warmed to 37°C, then coenzyme B_{12} (3 ml of a 120 μM solution of 5'-deoxyadenosyl -5, 6-dimethylbenzimadazoyl cobamide) added, and incubation continued in the dark for 2 hours at 37°C. Aldehyde formation was monitored by the MBTH assay. After 2 hours, 2, 4-dinitrophenylhydrazine (100 ml of a 0.4% solution in 3.6 N H_2SO_4) was added at room temperature causing an immediate yellow cloudiness, which rapidly coagulated to a precipitate. The

suspension was stood for 30 mins, with occasional shaking, then centrifuged at 5000 g for 10 mins. The liquor was decanted and the solid residue suspended in saturated brine (12 ml) then filtered and dried in vacuo to give a mixture of hydrazones. The mixture was heated with EtOH (5 ml) then filtered while hot. The filtrate was concentrated and separated by p.l.c. (Silica gel 0.5 mm; benzene) into 3, 3, 3-trifluoropropionaldehyde 2, 4-DNP (8.5 mg) and acetone 2, 4-DNP (8.5 mg). The aqueous liquor was extracted with CH_2Cl_2 (3 x 50 ml), and extracts were washed with saturated brine and dried. CH_2Cl_2 was removed in vacuo and the residue heated in benzene (100 ml) then filtered through silica gel (6 g) to remove 2, 4-DNP reagent, and yielded a mixture of acetone-2, 4-DNP and trifluoropropionaldehyde-2, 4-DNP. Recrystallisation gave 11 mg pure acetone-2, 4-DNP, and the residue was separated by p.l.c. (Silica gel 0.5 mm, benzene) to give a further 4 mg acetone-2, 4-DNP (total 23.5 mg) and 2.5 mg trifluoropropionaldehyde-2, 4-DNP (total 11 mg, 9.5%). Fractions were combined and recrystallised from EtOH.

3, 3, 3-Trifluoropropionaldehyde-2, 4-dinitrophenylhydrazone:

m.p. : 150 - 150.5°C (lit.[150] 150 - 150.8°C)

^{1}H n.m.r.
90 MHz ($CDCl_3$) : δ = 3.29 (octet, 6, 9, 2H, $CF_3\underline{CH_2}$), 7.49 (br t, 6, 1H, $-CH_2\underline{CH}=$), 7.96 (d, 10, 1H, H-6 Ar), 8.38 (d d, 10, 4, 1H, H-5 Ar), 9.13 (d, 3, 1H, H-3 Ar), 11.20 (br s, 1H, NH)

Acetone-2, 4-dinitrophenylhydrazone:

m.p. : 126 - 130°C (lit.[178] m.p. 128°C)

^{1}H n.m.r.
($CDCl_3$) : δ = 2.07 (s, 3H), 2.18 (s, 3H), 7.97 (d, 10, 1H), 8.35 (d d, 10, 3, 1H), 9.12 (d, 3, 1H).

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